(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 November 2003 (13.11.2003)

PCT

(10) International Publication Number
WO 03/092618 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US03/13817

(22) International Filing Date: 30 April 2003 (30.04.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/319,216 30 April 2002 (30.04.2002) US
60/319,313 12 June 2002 (12.06.2002) US

(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF SOUTH FLORIDA** [US/US]; 4202 East Fowler Avenue, FAO 126, Tampa, FL 33620-7900 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **MOHAPATRA, Shyam, S.** [US/US]; 18510 Country Crest Place, Tampa, FL 33647 (US). **BEHERA, Aruan, K.** [IN/US]; 750 Washington Street, Box 41, Boston, MA 02111 (US).

(74) Agents: **LADWIG, Glenn, P.** et al.; Saliwanchik, Lloyd & Saliwanchik, 2421 N.W. 41st Street, Suite A-1, Gainesville, FL 32606-6669 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/092618 A2

(54) Title: MATERIALS AND METHODS FOR PREVENTION AND TREATMENT OF RNA VIRAL DISEASES

(57) Abstract: The subject invention concerns a method of inhibiting an RNA virus infection within a patient by increasing the amount of 2-5 oligoadenylate (2-5 AS) activity within the patient. Preferably, the preventive and therapeutic methods of the present invention involve administering a nucleotide encoding 2-5 AS, or at least one catalytically active fragment thereof, such as the p40, p69, p100 subunits to a patient in need thereof. The present inventors have determined that overexpression of 2-5AS causes a reduction in epithelial cell damage, reduction in infiltration of mononuclear cells in the peribronchiolar and perivascular regions, and reduction in thickening of the septa in the lungs. Levels of chemokines, such as MIP1-α, are also reduced upon overexpression of 2-5AS. The subject invention also pertains to pharmaceutical compositions containing a nucleotide sequence encoding 2-5AS and pharmaceutically acceptable carrier, as well as vectors for delivery of the 2-5AS nucleotide sequence.

MATERIALS AND METHODS FOR PREVENTION AND TREATMENT OF RNA VIRAL DISEASES

Cross-Reference to Related Applications

This application claims the benefit of provisional patent application Serial No. 60/319,216, filed April 30, 2002, and provisional patent application Serial No. 60/319,313, filed June 12, 2002, which are hereby incorporated by reference in their entirety, including all nucleic acid sequences, amino acid sequences, figures, tables, and drawings.

Background of Invention

Respiratory syncytial virus (RSV) is a major respiratory pathogen in infants, young children, and the elderly, causing severe bronchiolitis, pneumonia, and exacerbation of asthma. In the United States alone, RSV causes approximately 4 million cases of respiratory tract infection annually, which results in 149,000 hospitalizations and 11,000 deaths. It has been established that interferon-gamma (IFN-γ) gene therapy is effective against RSV infection in BALB/c mice (Kumar *et al.*, Vaccine 18, 558-567, 1999).

Intranasal administration of a plasmid expressing IFN-γ cDNA proved to be an effective prophylaxis in mice. Furthermore, IFN-γ expressed by a recombinant respiratory syncytial virus attenuates virus replication in mice without compromising immunogenicity. IFN-γ, a type II interferon, is a pleotropic cytokine which plays an important role in modulating nearly all phases of immune and inflammatory responses. IFNs bind to specific receptors on cells and activate the JAK-STAT signaling cascade, which culminates in the transcriptional induction of IFN-stimulated genes (ISGs). The Jak1 and Jak2 phosphorylate STAT-1 following the binding of IFN-γ to its receptor. Once phosphorylated, STAT molecules dimerize and translocate to the nucleus and bind to gamma activated sequence (GAS) elements present in the regulatory regions of various ISGs. The antiviral mechanism of IFN-γ may involve one or more of a number of ISG-encoded products, including interferon regulatory factor-1 (IRF-1) double stranded RNA-dependent protein kinase (PKR), the Mx family of proteins, a family of 2'-5'-oligoadenylate synthetases (2-5 AS), and RNase L.

RNase L is constitutively expressed in most mammalian cells and is found in an inactive form bound to RNase L inhibitor (RLI), a 68kDa protein not regulated by IFN-γ. The 2-5 AS produces a series of 5' phosphorylated, 2', and 5'-linked oligoadenylates (2-5A) from ATP, when activated by double-stranded ribonucleic acid (dsRNA). Upon binding of 2-5 AS with RNase L, RLI is released and consequently, RNase L is dimerized and activated, mediating the cleavage of single-stranded RNA. However, the mechanism of the induction and activation of each of these genes is different in different cells and for the types of viruses. The mechanism of the IFN-γ-mediated anti-viral activity remains to be elucidated for many clinically important viruses.

Brief Summary of the Invention

The present invention provides materials and methods useful for inhibiting viral infections caused by ribonucleic acid (RNA) viruses that transiently produce double-stranded RNA during replication. The subject invention concerns therapeutic methods for preventing or decreasing the severity of symptoms associated with an RNA viral infection by increasing endogenous levels of 2'-5' oligoadenylate synthetase (2-5 AS) activity within the patient. Preferably, the endogenous levels of the 2-5 AS p40 subunit (*e.g.*, the 40kDa, 42kDa, 46kDa, or other splice variants), p69 subunit, (*e.g.*, the 69kDa, 71kDa, or other splice variants), p100 subunit, or combinations thereof, are increased within the patient.

The materials and methods of present invention are effective for treating or preventing human or animal infections from RNA viruses such as, members of the family paramyxoviridae, respiratory syncytial virus (RSV), Rhinovirus, Vaccinia, Reovirus, human immunodeficiency virus (HIV), encephalomyocarditis virus (EMCV), Hepatitis B, Hepatitis C, as well as bovine respiratory syncytial virus (BRSV), which infect cattle, sheep, and goats; Measles virus; Sendai virus; Parainfluenza 1, 2, and 3; Mumps virus, Simian virus; and Newcastle virus.

In one aspect, the method of the present invention involves the administration of a nucleotide sequence encoding 2-5 AS, or at least one catalytically active fragment of 2-5 AS, such as the p40, p69, or p100 subunits of 2-5 AS, to a patient in need thereof. The nucleotide sequence encoding 2-5 AS or at least one catalytically active fragment thereof can be administered to the patient, for example, in a viral vector or non-viral vector, such as plasmid deoxyribonucleic acid (DNA). In cases wherein the RNA virus is one which infects the patient's respiratory system, the nucleotide sequence encoding 2-5 AS, or at least one

catalytically active fragment thereof, is preferably administered orally or intranasally to the epithelial mucosa of the respiratory system.

The present invention also pertains to pharmaceutical compositions comprising a nucleotide sequence encoding 2-5 AS, or at least one catalytically active fragment thereof, such as the p40, p69, or p100 subunits of 2-5 AS, and a pharmaceutically acceptable carrier. The pharmaceutical compositions of the present invention are useful for preventing or decreasing the severity of symptoms associated with RNA viral infections. In another embodiment, the pharmaceutical compositions of the present invention comprise the 2-5 AS polypeptide, or at least one catalytically active fragment of the 2-5 AS polypeptide, and a pharmaceutically acceptable carrier. The pharmaceutical compositions of the present invention can include various agents that protect the nucleic acid or amino acid contents from protein degradation.

In another aspect, the present invention concerns vectors containing a nucleotide sequence encoding 2-5 AS, or at least one catalytically active fragment thereof, such as the p40, p69, or p100 subunits of 2-5 AS. Optionally, the vector can further include a promoter sequence operatively linked to the nucleotide encoding 2-5 AS or at least one catalytically active fragment thereof, permitting expression of the nucleotide sequence within a host cell. In another aspect, the present invention includes host cells that have been genetically modified with a nucleotide sequence encoding 2-5 AS, or at least one catalytically active fragment thereof, such as the p40, p69, or p100 subunits of 2-5 AS.

Brief Description of Drawings

Figures 1A-D show the results of pre-incubation of HEp-2 cells for 4-20 hours with different concentrations of IFN-γ and subsequent infection with RSV.

Figures 2A and 2B show results of a western blot analysis using specific antibodies to IRF-1, IRF-2, cytokeratin-18, double stranded RNA protein kinase (PKR), and inducible nitric oxide synthase (iNOS). Proteins were analyzed from cells at various time points post treatment with IFN-γ (1000 U/ml). Cytokeratin-18 was used as an internal control.

Figure 3 show results of northern analysis performed using gene specific probes for IRF-1 and the p40 and p69 isoforms of 2-5 AS.

Figure 4 shows results of exposure of HEp-2 cells to IFN-γ (1000 U/ml at 20 hours pre-infection) and treatment with equimolar mixtures of antisense oligonucleotides (ODNs)

to both p40 and p69 isoforms of 2-5 AS. Scrambled mismatch of the antisense ODN sequence to p40 and p69 at the same concentration were used as control.

Figures 5A-D show the results of northern analyses of (i) RNAs from RNAse L inhibitor (RLI) and HEp-2 using a gene specific probe for RLI and (ii) the level of mRNA expression of IRF-1, p40, and p69 isoforms of 2-5 AS.

Figure 6 show the results of treatment of both HEp-2 cells and RLI-14 cells with IFN-γ (at 100-1000 U/ml at 20 hours pre-infection) and subsequent infection with RSV.

Figure 7 shows the results of treatment of HEp-2 cells with IFN-γ (at 100-1000 U/ml at 20 hours pre-infection) and subsequent infection with RSV. 2-5A was transfected at 3 hours prior to RSV infection. Cells were harvested at 72 hours post infection and the clear cell homogenate was used for the RSV plaque assay (***: $p<0.005$; ††: $p<0.05$).

Figures 8A-8C show lung titers of RSV in infected mice following 2-5AS cDNA vaccination. BALB/c mice (n=4) were intranasally administered with p2'-5' AS (25 mg of DNA each time complexed with lipofectamine) or an equal amount of empty pVAX (CLONTECH, Palo Alto, CA, USA) vector DNA (as a transfection control) 3 times in 2 day intervals. As shown in Figure 8A, 2-5AS cDNA vaccination significantly attenuated lung titers of RSV. Figure 8B shows that vaccination with 2-5 AS cDNA decreases production of the chemokine macrophage inflammatory protein-1 alpha (MIP-1 α). The results of bronchoalveolar lavage (BAL) cell differential (Figure 8C) show that 2-5 AS does not significantly alter the cellular composition of the lung, although the percent of neutrophils is increased in the lungs of mice treated with 2-5 AS cDNA.

Figures 9A-9C show representative photomicrographs of lungs stained with hematoxylin and eosin (H & E). Figure 8A is an untreated control. Figures 9B and 9C show histological sections of RSV infected lungs following treatment with the empty pVAX vector and p2'-5' AS, respectively.

Figure 10 shows results of treatment with adenoviral vector (Ad)- 2-5AS (p40) results in attenuation of RSV replication. BALB/c mice were intranasally administered with Ad-p40 and then infected with RSV. Lungs were harvested five days post RSV infection and RSV replication was assayed by RT-PCR analysis of RSV- N gene. GAPDH was used as internal control.

Figure 11 shows that Ad-p40 attenuates RSV lung titers. Mice were intranasally given Ad-p40 and then infected with RSV. Lungs were harvested five days post RSV

infection and lung homogenate was used for RSV plaque assay. Ad-LacZ was used as control.

Figure 12 shows that Ad-p40 inhibits RSV induced airway reactivity. BALB/c mice were intranasally administered with Ad-p40 and subsequently infected with RSV. AHR was measured on day 4 post-RSV infection. Ad-p40 treatment significantly decreased pulmonary inflammation.

Figures 13A-13H show that Ad-p40 overexpression normalizes macrophage and lymphocyte numbers in the lung in RSV infected mice. BAL cell differential was performed and percentages of macrophage, lymphocytes and neutrophils was determined. Both Ad-IFNg and Ad-p40 treatment reduced the lymphocyte population to normal, compared to RSV-infected mice. Histological sections from lungs were stained with hematoxylin and eosin and representative photomicrographs are shown. Sections shown are as follows: Naive mice (Figure 13A; with magnified inset Figure 13B); RSV infected mice (Figure 13C; with magnified inset, Figure 13D); Ad-p40 treated mice (Figure 13E; with magnified inset, Figure 13F); and Ad-lacZ treated mice (Figure 13G; with magnified inset, Figure 13H).

Brief Description of Sequences

SEQ ID NO: 1 is a nucleotide coding sequence (CDS) for the human 40kDa splice variant of the 40/46kDa subunit ("p40 subunit") of 2'-5' oligoadenylate synthetase (National Center for Biotechnology Information (NCBI) Accession Number NM_016816).

SEQ ID NO: 2 is an amino acid sequence of the human 40kDa splice variant of the 40/46kDa subunit ("p40 subunit") of 2'-5' oligoadenylate synthetase (NCBI Accession Number NM_016816).

SEQ ID NO: 3 is a nucleotide coding sequence (CDS) for the human 46kDA splice variant of the 40/46kDa subunit ("p40 subunit") of 2'-5' oligoadenylate synthetase (National Center for Biotechnology Information (NCBI) Accession Number NM_016816).

SEQ ID NO: 4 is an amino acid sequence of the human 46kDA splice variant of the 40/46kDa subunit ("p40 subunit") of 2'-5' oligoadenylate synthetase (NCBI Accession Number NM_016816).

SEQ ID NO: 5 is a nucleotide coding sequence (CDS) for the human 69 kDA splice variant of the 69/71kDa subunit ("p69 subunit") of 2'-5' oligoadenylate synthetase (NCBI Accession Number NM_002535).

SEQ ID NO: 6 is an amino acid sequence of the human 69 kDa splice variant of the 69/71kDa subunit ("p69 subunit") of 2'-5' oligoadenylate synthetase (NCBI Accession Number NM_002535).

SEQ ID NO: 7 is a nucleotide coding sequence (CDS) for the human 71 kDA splice variant of the 69/71kDa subunit ("p69 subunit") of 2'-5' oligoadenylate synthetase (NCBI Accession Number NM_002535).

SEQ ID NO: 8 is an amino acid sequence of the human 71kDa splice variant of the 69/71kDa subunit ("p69 subunit") of 2'-5' oligoadenylate synthetase (NCBI Accession Number NM_002535).

SEQ ID NO: 9 is a nucleotide coding sequence (CDS) for the human 100kDa subunit ("p100 subunit") of 2'-5' oligoadenylate synthetase (NCBI Accession Number AF063613).

SEQ ID NO: 10 is an amino acid sequence of the human 100kDa subunit ("p100 subunit") of 2'-5' oligoadenylate synthetase (NCBI Accession Number AF063613).

SEQ ID NO: 11 is a nucleotide coding sequence (CDS) for the mouse homolog of the 2'-5' oligoadenylate synthetase 40kDa splice variant (p40 subunit) (NCBI Accession Number M33863).

SEQ ID NO: 12 is the amino acid sequence for the mouse homolog of the 2'-5' oligoadenylate synthetase 40kDa splice variant (p40 subunit) (NCBI Accession Number M33863).

SEQ ID NO: 13 is the human 2'-5' oligoadenylate synthetase 40/46kDa (p40 subunit) gene (NCBI Accession Number NM_016816).

SEQ ID NO: 14 is the human 2'-5' oligoadenylate synthetase 69/71kDa (p69 subunit) gene (NCBI Accession Number NM_002535).

SEQ ID NO: 15 is the human 2'-5' oligoadenylate synthetase 100kDa (p100 subunit) gene (NCBI Accession Number AF063613).

SEQ ID NO: 16 is the mouse homolog of the 2'-5' oligoadenylate synthetase 40kDa (p40 subunit) gene (NCBI Accession Number M33863).

SEQ ID NO: 17 is a phosphorothioate antisense oligonucleotide (ODN) designed against the p40 subunit of 2'-5' oligoadenylate synthetase.

SEQ ID NO: 18 is an ODN designed against the p69 subunit of 2'-5' oligoadenylate synthetase.

SEQ ID NO: 19 is a scramble of the antisense sequence to p40, *i.e.*, identical in base composition.

SEQ ID NO: 20 is a scramble of the antisense sequence to p69.

Detailed Disclosure of the Invention

The subject invention concerns a method of inhibiting an RNA virus infection within a patient by increasing the endogenous levels of 2-5 oligoadenylate synthetase (2-5 AS) activity within the patient. Preferably, the endogenous levels of the 2-5 AS p40 subunit (*e.g.*, 40kDa, 42kDA, 46kDa, or other splice variant), p69 subunit (*e.g.*, 69kDa, 71kDa, or other splice variant), p100 subunit, or combinations thereof, are increased within the patient.

The present inventors have determined that overexpression of the 2-5AS, or catalytically active fragments thereof, causes a reduction in epithelial cell damage, reduction in infiltration of mononuclear cells in the peribronchiolar and perivascular regions, and reduction in the thickening of the septa in the lungs of patients suffering from respiratory RNA viruses, such as respiratory syncytial virus (RSV). Levels of chemokines, such as MIP1-α, are also reduced upon overexpression of 2-5AS.

Infections from members of the family paramyxoviridae that produce double-stranded RNA as a requirement of replication can be prevented or treated using the present invention. Thus, infections by members of the genera paramyxovirus, morbillivirus, rubulavirus, pnuemovirus, and others can be inhibited in humans and animals. Examples of RNA viruses that produce double-stranded RNA during intermediate replication and which infect humans include, but are not limited to, respiratory syncytial virus (RSV), Rhinovirus, Vaccinia, Reovirus, HIV, EMCV, Hepatitis B, and Hepatitis C. Examples of RNA viruses that infect animals and produce double-stranded RNA during intermediate replication include, but are not limited to, bovine respiratory syncytial virus (BRSV), which infect cattle, sheep, and goats; Measles virus; Sendai virus; Parainfluenza 1, 2, and 3; Mumps virus, Simian virus; and Newcastle virus. Infections caused by coronavirus (such as that responsible for severe acute respiratory syndrome (SARS)), rotavirus, parainfluenza virus, West Nile virus, as well as other viruses in which interferon actively inhibits viral replication can be inhibited using the methods and compositions of the present invention.

In one aspect, the subject invention concerns a method of treating or preventing an RNA virus infection within a patient by increasing the *in vivo* concentration of 2-5 AS or a catalytically active fragment thereof within the patient, thereby inhibiting the RNA virus infection. Preferably, the methods of the present invention do not involve administration of interferon or a polynucleotide encoding interferon, such as interferon-alpha (IFN-α),

interferon-beta (IFN-β), or interferon-gamma (IFN-γ), or the administration of such IFN polypeptides. Thus, the methods and compositions of the present invention are directed to increasing the *in vivo* concentration of 2-5 AS or a catalytically active fragment thereof, which is an IFN-γ-induced downstream molecule. Advantageously, the methods and compositions of the present invention exhibit an antiviral effect without the adverse effects associated with IFN-γ.

The *in vivo* concentration of the 2-5 AS, or a catalytically active fragment thereof, can be increased, for example, by exogenous administration of the 2-5 AS polypeptide, or a catalytically active fragment of the polypeptide. Preferably, the *in vivo* concentration of the 2-5 AS polypeptide or catalytically active fragment is increased by increasing or up-regulating the functional expression of the nucleotide sequence encoding 2-5 AS or at least one catalytically active fragment thereof, such as the p40, p69, or p100 subunits, as gene therapy. More preferably, a nucleotide sequence encoding 2-5 AS or at least one catalytically active fragment thereof can be administered to a patient and expressed in order to increase the endogenous level of 2-5 AS enzymatic activity within the patient. For example, at least one nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 14, 15, 16, and 17, or a catalytically active fragment thereof, can be administered to the patient. The nucleotide sequence can be administered to a patient's cells *in vivo* or *in vitro* (including *ex vivo*, genetically modifying the patient's own cells *ex vivo* and subsequently administering the modified cells back into the patient).

In another aspect of the invention, 2-5 AS polypeptide, or at least one catalytically active fragment thereof, is administered to a patient in order to increase the antiviral function of 2-5 AS within the patient. Preferably, the polypeptides utilized are those disclosed herein. The polypeptides can comprise catalytically active fragments of the full-length 2-5 AS amino acid sequence, such as the p40, p69, or p100 subunits, including splice variants of these subunits, or mammalian homologs of these subunits (*e.g.*, the p46 isoform of OAS-1; accession number NP_058132.1), such as murine homologs. For example, the polypeptides can comprise one or more amino acid sequences set forth herein as SEQ ID NOs: 2, 4, 6, 8, 10, 12, 13, 14, 15 or 16, or catalytically active fragments of these amino acid sequences.

Various means for delivering polypeptides to a cell can be utilized to carry out the methods of the subject invention. For example, protein transduction domains (PTDs) can be fused to the polypeptide, producing a fusion polypeptide, in which the PTDs are capable of transducing the polypeptide cargo across the plasma membrane (Wadia, J.S. and Dowdy,

S.F., *Curr. Opin. Biotechnol.*, 2002, 13(1)52-56). Examples of PTDs include the Drosophila homeotic transcription protein antennapedia (Antp), the herpes simples virus structural protein VP22, and the human immuno-deficiency virus 1 (HIV-1) transcriptional activator Tat protein.

According to the method of RNA virus inhibition of the subject invention, recombinant cells can be administered to a patient, wherein the recombinant cells have been genetically modified to express the gene encoding 2-5 AS or at least one catalytically active fragment thereof, such as the p40, p69, or p100 subunits of 2-5 AS. If the cells to be genetically modified already express a gene encoding 2-5 AS, the genetic modification can serve to enhance or increase expression of the gene encoding 2-5 AS or a catalytically active fragment of 2-5 AS beyond the normal or constitutive amount (*e.g.*, "overexpression").

The method of RNA virus inhibition of the subject invention can be used to treat a patient suffering from an RNA virus infection, or as a preventative of RNA virus infection (*i.e.*, prophylactic treatment). According to the methods of the subject invention, various other compounds, such as antiviral compounds, can be administered in conjunction with (before, during, or after) increasing the *in vivo* concentrations of 2-5 AS or at least one catalytically active fragment within the patient. Various compositions and methods for preventing or treating RNA virus infection can be used in conjunction with the compositions and methods of the subject invention, such as those described in U.S. Patent No. 6,489,306, filed February 23, 1999, and U.S. published patent application Serial No. 2003/00068333, filed February 12, 2002, which are incorporated herein by reference in their entirety. For example, nucleotide sequences encoding 2-5 AS or at least one catalytically active fragment thereof can be conjugated with chitosan, a biodegradable, human-friendly cationic polymer that increases mucosal absorption of the gene expression vaccine without any adverse effects, as described in U.S. published patent application Serial No. 2003/00068333.

The nucleotide sequence can be formulated in the form of nanospheres with chitosan. Chitosan allows increased bioavailability of the DNA because of protection from degradation by serum nucleases in the matrix and thus has great potential as a mucosal gene delivery system, for example. Chitosan exhibits various beneficial effects, such as anticoagulant activity, wound-healing properties, and immunostimulatory activity, and is capable of modulating immunity of the mucosa and bronchus-associated lymphoid tissue.

Nucleotide, polynucleotide, or nucleic acid sequences(s) are understood to mean, according to the present invention, either a double-stranded DNA, a single-stranded DNA,

products of transcription of the said DNAs (*e.g.*, RNA molecules), or corresponding RNA molecules that are not products of transcription. It should also be understood that the present invention does not relate to the genomic nucleotide sequences encoding 2-5 AS or catalytically active fragments thereof in their natural/native environment or natural/native state. The nucleic acid, polynucleotide, or nucleotide sequences of the invention have been isolated, purified (or partially purified), by separation methods including, but not limited to, ion-exchange chromatography, molecular size exclusion chromatography, affinity chromatography, or by genetic engineering methods such as amplification, cloning or subcloning.

Optionally, the polynucleotide sequence encoding 2-5 AS or catalytically active fragment thereof can also contain one or more polynucleotides encoding heterologous polypeptide sequences (*e.g.*, tags that facilitate purification of the polypeptides of the invention (see, for example, U.S. Patent No. 6,342,362, hereby incorporated by reference in its entirety; Altendorf *et al.* [1999-WWW, 2000] "Structure and Function of the F_o Complex of the ATP Synthase from *Escherichia Coli*," *J. of Experimental* Biology 203:19-28, The Co. of Biologists, Ltd., G.B.; Baneyx [1999] "Recombinant Protein Expression in *Escherichia coli*," Biotechnology 10:411-21, Elsevier Science Ltd.; Eihauer *et al.* [2001] "The FLAG Peptide, a Versatile Fusion Tag for the Purification of Recombinant Proteins," *J. Biochem Biophys Methods* 49:455-65; Jones *et al.* [1995] *J. Chromatography* 707:3-22; Jones *et al.* [1995] "Current Trends in Molecular Recognition and Bioseparation," *J. of Chromatography A.* 707:3-22, Elsevier Science B.V.; Margolin [2000] "Green Fluorescent Protein as a Reporter for Macromolecular Localization in Bacterial Cells," *Methods* 20:62-72, Academic Press; Puig *et al.* [2001] "The Tandem Affinity Purification (TAP) Method: A General Procedure of Protein Complex Purification," *Methods* 24:218-29, Academic Press; Sassenfeld [1990] "Engineering Proteins for Purification," TibTech 8:88-93; Sheibani [1999] "Prokaryotic Gene Fusion Expression Systems and Their Use in Structural and Functional Studies of Proteins," *Prep. Biochem. & Biotechnol.* 29(1):77-90, Marcel Dekker, Inc.; Skerra *et al.* [1999] "Applications of a Peptide Ligand for Streptavidin: the *Strep*-tag", *Biomolecular Engineering* 16:79-86, Elsevier Science, B.V.; Smith [1998] "Cookbook for Eukaryotic Protein Expression: Yeast, Insect, and Plant Expression Systems," *The* Scientist 12(22):20; Smyth *et al.* [2000] "Eukaryotic Expression and Purification of Recombinant Extracellular Matrix Proteins Carrying the Strep II Tag", *Methods in Molecular Biology*, 139:49-57; Unger [1997] "Show Me the Money: Prokaryotic Expression Vectors and

Purification Systems," *The Scientist* 11(17):20, each of which is hereby incorporated by reference in their entireties), or commercially available tags from vendors such as such as STRATAGENE (La Jolla, CA), NOVAGEN (Madison, WI), QIAGEN, Inc., (Valencia, CA), or INVITROGEN (San Diego, CA).

Other aspects of the invention provide vectors containing one or more of the polynucleotides of the invention, such as vectors containing nucleotides encoding 2-5 AS or catalytically active fragments of 2-5 AS, such as the p40 and/or p69 subunits. The vectors can be vaccine, replication, or amplification vectors. In some embodiments of this aspect of the invention, the polynucleotides are operably associated with regulatory elements capable of causing the expression of the polynucleotide sequences. Such vectors include, among others, chromosomal, episomal and virus-derived vectors, *e.g.*, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, lentiviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations of the aforementioned vector sources, such as those derived from plasmid and bacteriophage genetic elements (*e.g.*, cosmids and phagemids). Preferably, the vector is an adenoaviral vector or adeno-associated virus vector.

As indicated above, vectors of this invention can also comprise elements necessary to provide for the expression and/or the secretion of 2-5 AS, or a catalytically active fragment thereof, encoded by the nucleotide sequences of the invention in a given host cell. The vector can contain one or more elements selected from the group consisting of a promoter sequence, signals for initiation of translation, signals for termination of translation, and appropriate regions for regulation of transcription. In certain embodiments, the vectors can be stably maintained in the host cell and can, optionally, contain signal sequences directing the secretion of translated protein. Other embodiments provide vectors that are not stable in transformed host cells. Vectors can integrate into the host genome or be autonomously-replicating vectors.

In a specific embodiment, a vector comprises a promoter operably linked to a 2-5 AS-encoding nucleic acid sequence (or a catalytically active fragment thereof), one or more origins of replication, and, optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene). Non-limiting exemplary vectors for the expression of the polypeptides of the invention include pBr-type vectors, pET-type plasmid vectors (PROMEGA), pBAD

plasmid vectors (INVITROGEN), and pVAX plasmid vectors (INVITROGEN), or others provided in the examples below. Furthermore, vectors according to the invention are useful for transforming host cells for the cloning or expression of the nucleotide sequences of the invention.

Promoters which may be used to control expression include, but are not limited to, the CMV promoter, the SV40 early promoter region (Bernoist and Chambon [1981] *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.* [1980] *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner *et al.* [1981] *Proc. Natl. Acad. Sci. USA* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al.* [1982] *Nature* 296:39-42); prokaryotic vectors containing promoters such as the β-lactamase promoter (Villa-Kamaroff, *et al.* [1978] *Proc. Natl. Acad. Sci. USA* 75:3727-3731), or the *tac* promoter (DeBoer, *et al.* [1983] *Proc. Natl. Acad. Sci. USA* 80:21-25); the lung specific promoters such as surfactant protein B promoter (Venkatesh *et al.*, *Am. J. Physiol.* 268 (*Lung Cell Mol. Physiol.* 12):L674-L682, 1995); see also, "Useful Proteins from Recombinant Bacteria" in *Scientific American*, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella *et al.* [1983] *Nature* 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, *et al.* [1981] *Nucl. Acids Res.* 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella *et al.* [1984] *Nature* 310:115-120); promoter elements from yeast or fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, and/or the alkaline phosphatase promoter.

The subject invention also provides for "homologous" or "modified" nucleotide sequences. Modified nucleic acid sequences will be understood to mean any nucleotide sequence obtained by mutagenesis according to techniques well known to persons skilled in the art, and exhibiting modifications in relation to the normal sequences. For example, mutations in the regulatory and/or promoter sequences for the expression of a polypeptide that result in a modification of the level of expression of a polypeptide according to the invention provide for a "modified nucleotide sequence". Likewise, substitutions, deletions, or additions of nucleic acid to the polynucleotides of the invention provide for "homologous" or "modified" nucleotide sequences. In various embodiments, "homologous" or "modified" nucleic acid sequences have substantially the same biological activity as the native (naturally occurring) 2-5 AS or subunit thereof. A "homologous" or "modified" nucleotide sequence

will also be understood to mean a subunit or a splice variant of the polynucleotides of the instant invention or any nucleotide sequence encoding a "modified polypeptide" as defined below.

A homologous nucleotide sequence, for the purposes of the present invention, encompasses a nucleotide sequence having a percentage identity with the bases of the nucleotide sequences of between at least (or at least about) 20.00% to 99.99% (inclusive), and which encodes a catalytically active polypeptide. The aforementioned range of percent identity is to be taken as including, and providing written description and support for, any fractional percentage, in intervals of 0.01%, between 20.00% and 99.99%. These percentages are purely statistical and differences between two nucleic acid sequences can be distributed randomly and over the entire sequence length.

In various embodiments, homologous sequences exhibiting a percentage identity with the bases of the nucleotide sequences of the present invention can have 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity with the polynucleotide sequences of the instant invention.

Both protein and nucleic acid sequence homologies may be evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman [1988] *Proc. Natl. Acad. Sci. USA* 85(8):2444-2448; Altschul *et al.* [1990] *J. Mol. Biol.* 215(3):403-410; Thompson *et al.* [1994] *Nucleic Acids Res.* 22(2):4673-4680; Higgins *et al.* [1996] *Methods Enzymol.* 266:383-402; Altschul *et al.* [1990] *J. Mol. Biol.* 215(3):403-410; Altschul *et al.* [1993] *Nature Genetics* 3:266-272).

Nucleotide sequences encoding polypeptides with enhanced 2-5 AS catalytic activity can be obtained by "gene shuffling" (also referred to as "directed evolution", and "directed mutagenesis"), and used in the compositions and methods of the present invention. Gene shuffling is a process of randomly recombining different sequences of functional genes (recombining favorable mutations in a random fashion) (U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; and 5,837,458). Thus, protein engineering can be accomplished by gene shuffling, random complex permutation sampling, or by rational design based on three-

dimensional structure and classical protein chemistry (Cramer *et al.*, *Nature*, 391:288-291, 1998; and Wulff *et al.*, *The Plant Cell*, 13:255-272, 2001).

The subject invention also provides nucleotide sequences complementary to any of the polynucleotide sequences disclosed herein. Thus, the invention is understood to include any DNA whose nucleotides are complementary to those of 2-5 AS polynucleotide sequence of the invention, or catalytically active fragments thereof, and whose orientation is reversed (*e.g.*, an antisense sequence).

The present invention further provides catalytically active fragments of the 2-5 AS polynucleotide sequences, including catalytically active fragments of the 2-5 AS subunit nucleotide sequences, provided herein. Representative fragments of the polynucleotide sequences according to the invention will be understood to mean any nucleotide fragment having at least 8 or 9 successive nucleotides, preferably at least 12 successive nucleotides, and still more preferably at least 15 or at least 20 successive nucleotides of the sequence from which it is derived. The upper limit for such fragments is the total number of polynucleotides found in the full-length sequence (or, in certain embodiments, of the full length open reading frame (ORF) identified herein). It is understood that, optionally, such fragments refer only to portions of the disclosed polynucleotide sequences that are not listed in a publicly available database or prior art references. However, it should be understood that with respect to the method for inhibiting RSV of the subject invention, disclosed nucleotides (and polypeptides encoded by such nucleotides) that are listed in a publicly available database or prior art reference can also be utilized. For example, nucleotide sequences that are 2-5 AS p40 or p69 subunit homologs, or fragments thereof, which have been previously identified, can be utilized to carry out the method for inhibiting RNA virus infection of the subject invention.

In other embodiments, fragments contain from one nucleotide less than the full length 2-5 AS enzyme, or from one nucleotide less than a catalytically active subunit thereof, such as p40 or p69 subunit polynucleotide CDS sequences (*e.g.*, 1,203 and 1,207 nucleotides for the 40kDa splice variant and 46kDa splice variant, respectively; and 2063 and 2,168 nucleotides for the 69kDa splice variant and 71kDa splice variant, respectively) to fragments containing the smallest number of nucleotides encoding a polypeptide that retains at least some 2-5 AS enzymatic activity.

Among these representative fragments, those capable of hybridizing under stringent conditions with a nucleotide sequence encoding 2-5 AS or subunits thereof are preferred. Conditions of high or intermediate stringency are provided *infra* and are chosen to allow for

hybridization between two complementary DNA fragments. Hybridization conditions for a polynucleotide of about 1,000 to 3,000 bases in size will be adapted by persons skilled in the art for larger- or smaller-sized oligonucleotides, according to methods well known in the art (see, for example, Sambrook *et al.* [1989] *Molecular Cloning, A Laboratory Manual, Second Edition*, Cold Spring Harbor Press, N.Y., pp. 9.47-9.57).

The subject invention also provides detection probes (*e.g.*, fragments of the disclosed polynucleotide sequences) for hybridization with a target sequence or an amplicon generated from the target sequence. Such a detection probe will advantageously have as sequence a sequence of at least 9, 12, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 nucleotides. Alternatively, detection probes can comprise 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127 and up to, for example, 1,203 consecutive nucleotides, 1,207 consecutive nucleotides, 2,064 consecutive nucleotides, 2,186 consecutive nucleotides, 3,264 consecutive nucleotides, and 1,104 consecutive nucleotides of those disclosed herein, which correspond, respectively, to the human 40kDa splice variant of the 2-5AS p40 subunit (SEQ ID NO:1), human 46kDa splice variant 2-5AS p40 subunit (SEQ ID NO:3), human 69kDa splice variant of the 2-5AS p69 subunit (SEQ ID NO:5), human 71kDa splice variant of the 2-5AS p69 subunit (SEQ ID NO:7), human p100 subunit (SEQ ID NO:9), and the mouse homolog of the 2-5AS p40 subunit (SEQ ID NO:11). The detection probes can also be used as labeled probe or primer in the subject invention. Labeled probes or primers are labeled with a radioactive compound or with another type of label. Alternatively, non-labeled nucleotide sequences may be used directly as probes or primers; however, the sequences are generally labeled with a radioactive element (^{32}P, ^{35}S, ^{3}H, ^{125}I) or with a molecule such as biotin, acetylaminofluorene, digoxigenin, 5-bromo-deoxyuridine, or fluorescein to provide probes that can be used in numerous applications.

The nucleotide sequences according to the invention may also be used in analytical systems, such as DNA chips. DNA chips and their uses are well known in the art and (see for example, U.S. Patent Nos. 5,561,071; 5,753,439; 6,214,545; Schena *et al.* [1996] *BioEssays* 18:427-431; Bianchi *et al.* [1997] *Clin. Diagn. Virol.* 8:199-208; each of which is hereby

incorporated by reference in their entireties) and/or are provided by commercial vendors such as AFFYMETRIX, Inc. (Santa Clara, CA).

Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for duplex formation. Severity of conditions can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak [1987] *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

By way of example, hybridization of immobilized DNA on Southern blots with ^{32}P-labeled gene-specific probes can be performed by standard methods (Maniatis *et al.* [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York). In general, hybridization and subsequent washes can be carried out under moderate to high stringency conditions that allow for detection of target sequences with homology to the exemplified polynucleotide sequence. For double-stranded DNA gene probes, hybridization can be carried out overnight at 20-25°C below the melting temperature (Tm) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz *et al.* [1983] *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

T_m=81.5°C+16.6 Log[Na+]+0.41(%G+C)-0.61(% formamide)-600/length of duplex in base pairs.

Washes are typically carried out as follows:

(1) twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash);

(2) once at T_m-20°C for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

For oligonucleotide probes, hybridization can be carried out overnight at 10-20°C below the melting temperature (T_m) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. T_m for oligonucleotide probes can be determined by the following formula:

T_m (°C)=2(number T/A base pairs) + 4(number G/C base pairs) (Suggs *et al.* [1981] *ICN-UCLA Symp. Dev. Biol. Using Purified Genes*, D.D. Brown [ed.], Academic Press, New York, 23:683-693).

Washes can be carried out as follows:

(1) twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash;

2) once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment >70 or so bases in length, the following conditions can be used:

Low:	1 or 2X SSPE, room temperature
Low:	1 or 2X SSPE, 42°C
Moderate:	0.2X or 1X SSPE, 65°C
High:	0.1X SSPE, 65°C.

By way of another non-limiting example, procedures using conditions of high stringency can also be performed as follows: Pre-hybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C, the preferred hybridization temperature, in pre-hybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Alternatively, the hybridization step can be performed at 65°C in the presence of SSC buffer, 1 x SSC corresponding to 0.15M NaCl and 0.05 M Na citrate. Subsequently, filter washes can be done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA, followed by a wash in 0.1X SSC at 50°C for 45 min. Alternatively, filter washes can be performed in a solution containing 2 x SSC and 0.1% SDS, or 0.5 x SSC and 0.1% SDS, or 0.1 x SSC and 0.1% SDS at 68°C for 15 minute intervals. Following the wash steps, the hybridized probes are detectable by autoradiography. Other conditions of high stringency which may be used are well known in the art (see, for example, Sambrook *et al.* [1989] *Molecular Cloning, A Laboratory Manual, Second Edition*, Cold Spring Harbor Press, N.Y., pp. 9.47-9.57; and Ausubel *et al.* [1989] *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y., each incorporated herein in its entirety).

A further non-limiting example of procedures using conditions of intermediate stringency are as follows: Filters containing DNA are pre-hybridized, and then hybridized at a temperature of 60°C in the presence of a 5 x SSC buffer and labeled probe. Subsequently, filters washes are performed in a solution containing 2x SSC at 50°C and the hybridized probes are detectable by autoradiography. Other conditions of intermediate stringency which may be used are well known in the art (see, for example, Sambrook *et al.* [1989] *Molecular Cloning, A Laboratory Manual, Second Edition*, Cold Spring Harbor Press, N.Y., pp. 9.47-9.57; and Ausubel *et al.* [1989] *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y., each of which is incorporated herein in its entirety).

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probe sequences of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions and deletions can be produced in a given polynucleotide sequence in many ways, and these methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

It is also well known in the art that restriction enzymes can be used to obtain functional fragments of the subject DNA sequences. For example, *Bal*31 exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for example, Maniatis *et al.* [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York; Wei *et al.* [1983] *J. Biol. Chem.* 258:13006-13512. The nucleic acid sequences of the subject invention can also be used as molecular weight markers in nucleic acid analysis procedures.

The invention also provides host cells transformed by a polynucleotide according to the invention and the production of 2-5 AS or a catalytically active fragment thereof, by the transformed host cells. In some embodiments, transformed cells comprise an expression vector containing 2-5 AS nucleotide sequences or a catalytically active fragment thereof. Other embodiments provide for host cells transformed with nucleic acids. Yet other embodiments provide transformed cells comprising an expression vector containing fragments of 2-5 AS p40 and/or p69 subunit nucleotide sequences. Transformed host cells according to the invention are cultured under conditions allowing the replication and/or the expression of the 2-5 AS nucleotide sequence or a catalytically active fragment thereof, such

as the p40 and/or p69 subunits. Expressed polypeptides are recovered from culture media and purified, for further use, according to methods known in the art.

The host cell may be chosen from eukaryotic or prokaryotic systems, for example bacterial cells (Gram negative or Gram positive), yeast cells, animal cells, human cells, plant cells, and/or insect cells using baculovirus vectors. In some embodiments, the host cell for expression of the polypeptides include, and are not limited to, those taught in U.S. Patent Nos. 6,319,691; 6,277,375; 5,643,570; 5,565,335; Unger [1997] *The Scientist* 11(17):20; or Smith [1998] *The Scientist* 12(22):20, each of which is incorporated by reference in its entirety, including all references cited within each respective patent or reference. Other exemplary, and non-limiting, host cells include *Staphylococcus* spp., *Enterococcus* spp., *E. coli*, and *Bacillus subtilis*; fungal cells, such as *Streptomyces* spp., *Aspergillus* spp., *S. cerevisiae*, *Schizosaccharomyces pombe*, *Pichia pastoris*, *Hansela polymorpha*, *Kluveromyces lactis*, and *Yarrowia lipolytica*; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells. A great variety of expression systems can be used to produce the 2-5 AS polypeptides or catalytically active fragments thereof and encoding polynucleotides can be modified according to methods known in the art to provide optimal codon usage for expression in a particular expression system.

Furthermore, a host cell strain may be chosen that modulates the expression of the inserted sequences, modifies the gene product, and/or processes the gene product in the specific fashion. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, phosphorylation) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product whereas expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to provide "native" glycosylation of a heterologous protein. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

Nucleic acids and/or vectors encoding 2-5 AS, or catalytically active fragments thereof, such as the p40 and/or p69 subunits, can be introduced into host cells by well-known

methods, such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection (see, for example, Sambrook *et al.* [1989] *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

The subject invention also provides for the expression of the 2-5 AS p40 or p69 subunit, derivative, or a analogue (*e.g.*, a splice variant) encoded by a polynucleotide sequence disclosed herein. Alternatively, the invention provides for the expression of a polynucleotide encoding a fragment of a 2-5 AS p40 or p69 subunit. In either embodiment, the disclosed sequences can be regulated by a second nucleic acid sequence so that the polypeptide or fragment is expressed in a host transformed with a recombinant DNA molecule according to the subject invention. For example, expression of a protein or peptide may be controlled by any promoter/enhancer element known in the art.

In the context of the instant invention, the terms polypeptide, peptide and protein are used interchangeably. Likewise, the terms analogue and homologous are also used interchangeably. It should be understood that the invention does not relate to the polypeptides in natural form or native environment. Peptides and polypeptides according to the invention have been isolated or obtained by purification from natural sources (or their native environment), chemically synthesized, or obtained from host cells prepared by genetic manipulation (*e.g.*, the polypeptides, or fragments thereof, are recombinantly produced by host cells). Polypeptides according to the instant invention may also contain non-natural amino acids, as will be described below.

"Analogues" or "homologous" polypeptides will be understood to designate the polypeptides containing, in relation to the native polypeptide, modifications such as deletion, addition, or substitution of at least one amino acid, truncation, extension, or the addition of chimeric heterologous polypeptides. Optionally, "analogues" or "homologous" polypeptides can contain a mutation or post-translational modifications. Among the "analogues" or "homologous" polypeptides, those whose amino acid sequence exhibits 20.00% to 99.99% (inclusive) identity to the native polypeptide sequence are preferred. The aforementioned range of percent identity is to be taken as including, and providing written description and support for, any fractional percentage, in intervals of 0.01%, between 50.00% and, up to, including 99.99%. These percentages are purely statistical and differences between two polypeptide sequences can be distributed randomly and over the entire sequence length.

"Analogues" or "homologous" polypeptide sequences exhibiting a percentage identity with the human 2-5 AS polypeptides, or subunits thereof, can alternatively have 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 91, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity with the polypeptide sequences of the instant invention. The expression equivalent amino acid is intended here to designate any amino acid capable of being substituted for one of the amino acids in the basic structure without, however, essentially modifying the biological activities of the corresponding peptides and as provided below.

By way of example, amino acid substitutions can be carried out without resulting in a substantial modification of the biological activity of the corresponding modified polypeptides; for example, the replacement of leucine with valine or isoleucine; aspartic acid with glutamic acid; glutamine with asparagine; arginine with lysine; and the reverse substitutions can be performed without substantial modification of the biological activity of the polypeptides.

The subject invention also provides catalytically active fragments of the 2-5 AS polypeptide, and catalytically active fragments of the 2-5 AS subunits, according to the invention, which are capable of eliciting an immune response against RSV. The immune response can provide components (either antibodies or components of the cellular immune response (*e.g.*, B-cells, helper, cytotoxic, and/or suppressor T-cells) reactive with the catalytically active fragment of the polypeptide, the intact, full length, unmodified polypeptide, or both the catalytically active fragment of the polypeptide and the intact, full length, unmodified polypeptides.

Catalytically active fragments according to the invention can comprise from five (5) amino acids to one amino acid less than the full length of any polypeptide sequence provided herein. For example, fragments comprising 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, and up to one amino acid less than the full length amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12, are provided herein.

Fragments, as described herein, can be obtained by cleaving the polypeptides of the invention with a proteolytic enzyme (such as trypsin, chymotrypsin, or collagenase) or with a

chemical reagent, such as cyanogen bromide (CNBr). Alternatively, polypeptide fragments can be generated in a highly acidic environment, for example at pH 2.5. Such polypeptide fragments may be equally well prepared by chemical synthesis or using hosts transformed with an expression vector containing nucleic acids encoding polypeptide fragments according to the invention. The transformed host cells contain a nucleic acid and are cultured according to well-known methods; thus, the invention allows for the expression of these fragments, under the control of appropriate elements for regulation and/or expression of the polypeptide fragments.

Modified polypeptides according to the invention are understood to designate a polypeptide obtained by variation in the splicing of transcriptional products of the 2-5 AS gene, genetic recombination, or by chemical synthesis as described below. Modified polypeptides contain at least one modification in relation to the normal polypeptide sequence. These modifications can include the addition, substitution, deletion of amino acids contained within the polypeptides of the invention.

Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the polypeptide. For example, the class of nonpolar amino acids include Ala, Val, Leu, Ile, Pro, Met, Phe, and Trp; the class of uncharged polar amino acids includes Gly, Ser, Thr, Cys, Tyr, Asn, and Gln; the class of acidic amino acids includes Asp and Glu; and the class of basic amino acids includes Lys, Arg, and His. In some instances, non-conservative substitutions can be made where these substitutions do not significantly detract from the biological activity of the polypeptide.

In order to extend the life of the polypeptides of the invention, it may be advantageous to use non-natural amino acids, for example in the D form, or alternatively amino acid analogs, such as sulfur-containing forms of amino acids. Alternative means for increasing the life of polypeptides can also be used in the practice of the instant invention. For example, polypeptides of the invention, and fragments thereof, can be recombinantly modified to include elements that increase the plasma, or serum half-life of the polypeptides of the invention. These elements include, and are not limited to, antibody constant regions (see for example, U.S. Patent No. 5,565,335, hereby incorporated by reference in its entirety, including all references cited therein), or other elements such as those disclosed in U.S. Patent Nos. 6,319,691; 6,277,375; or 5,643,570, each of which is incorporated by reference in its entirety, including all references cited within each respective patent. Alternatively, the 2-5

AS polynucleotides, or catalytically active fragments thereof, used in the instant invention can be recombinantly fused to elements that are useful in the preparation of immunogenic constructs for the purposes of vaccine formulation or elements useful for the isolation of the polypeptides of the invention.

The polypeptides, fragments, and immunogenic fragments of the invention may further contain linkers that facilitate the attachment of the fragments to a carrier molecule for delivery or diagnostic purposes. The linkers can also be used to attach fragments according to the invention to solid support matrices for use in affinity purification protocols. In this aspect of the invention, the linkers specifically exclude, and are not to be considered anticipated, where the fragment is a subsequence of another peptide, polypeptide, or protein as identified in a search of protein sequence databases as indicated in the preceding paragraph. In other words, the non-identical portions of the other peptide, polypeptide, or protein is not considered to be a "linker" in this aspect of the invention. Non-limiting examples of "linkers" suitable for the practice of the invention include chemical linkers (such as those sold by Pierce, Rockford, IL), peptides that allow for the connection of the immunogenic fragment to a carrier molecule (see, for example, linkers disclosed in U.S. Patent Nos. 6,121,424; 5,843,464; 5,750,352; and 5,990,275, hereby incorporated by reference in their entirety). In various embodiments, the linkers can be up to 50 amino acids in length, up to 40 amino acids in length, up to 30 amino acids in length, up to 20 amino acids in length, up to 10 amino acids in length, or up to 5 amino acids in length.

In other specific embodiments, the 2-5 AS polypeptide or 2-5 AS subunit polypeptide, peptides, derivatives, or analogs thereof may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (*e.g.*, a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art (see, for example, U.S. Patent No. 6,342,362, hereby incorporated by reference in its entirety; Altendorf *et al.* [1999-WWW, 2000] "Structure and Function of the F_o Complex of the ATP Synthase from *Escherichia Coli*," *J. of Experimental* Biology 203:19-28, The Co. of Biologists, Ltd., G.B.; Baneyx [1999] "Recombinant Protein Expression in *Escherichia coli*," *Biotechnology* 10:411-21, Elsevier Science Ltd.; Eihauer *et al.* [2001] "The FLAG Peptide, a Versatile Fusion Tag for the Purification of Recombinant Proteins," *J. Biochem Biophys Methods*

49:455-65; Jones *et al.* [1995] *J. Chromatography* 707:3-22; Jones *et al.* [1995] "Current Trends in Molecular Recognition and Bioseparation," *J. Chromatography A.* 707:3-22, Elsevier Science B.V.; Margolin [2000] "Green Fluorescent Protein as a Reporter for Macromolecular Localization in Bacterial Cells," *Methods* 20:62-72, Academic Press; Puig *et al.* [2001] "The Tandem Affinity Purification (TAP) Method: A General Procedure of Protein Complex Purification," *Methods* 24:218-29, Academic Press; Sassenfeld [1990] "Engineering Proteins for Purification," *TibTech* 8:88-93; Sheibani [1999] "Prokaryotic Gene Fusion Expression Systems and Their Use in Structural and Functional Studies of Proteins," *Prep. Biochem. & Biotechnol.* 29(1):77-90, Marcel Dekker, Inc.; Skerra *et al.* [1999] "Applications of a Peptide Ligand for Streptavidin: The *Strep*-tag", *Biomolecular Engineering* 16:79-86, Elsevier Science, B.V.; Smith [1998] "Cookbook for Eukaryotic Protein Expression: Yeast, Insect, and Plant Expression Systems," *The* Scientist 12(22):20; Smyth *et al.* [2000] "Eukaryotic Expression and Purification of Recombinant Extracellular Matrix Proteins Carrying the Strep II Tag", *Methods in Molecular Biology*, 139:49-57; Unger [1997] "Show Me the Money: Prokaryotic Expression Vectors and Purification Systems," *The Scientist* 11(17):20, each of which is hereby incorporated by reference in their entireties). Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. Fusion peptides can comprise polypeptides of the subject invention and one or more protein transduction domains, as described above. Such fusion peptides are particularly useful for delivering the cargo polypeptide through the cell membrane.

Increasing the amount of 2-5 AS enzymatic activity (*e.g.*, p40, p69, and/or p100 subunit activity) within a tissue is useful in preventing an RNA virus infection, or in treating an existing RNA virus infection. Thus, according to the methods of the subject invention, the amount of 2-5 AS activity can be increased within a tissue by directly administering the 2-5 AS polypeptide or a catalytically active fragment thereof to a patient suffering from or susceptible to an RNA virus infection (such as exogenous delivery of the 2-5 AS p40, p69, and/or p100 subunit polypeptide) or by indirect or genetic means (such as delivery of a nucleotide sequence encoding the 2-5 AS polypeptide or a catalytically active fragment thereof, or upregulating the endogenous 2-5 AS polypeptide activity).

As used herein, the term "administration" or "administering" refers to the process of delivering an agent to a patient, wherein the agent directly or indirectly increases 2-5 AS enzymatic function within the patient and, preferably, at the target site. The process of

administration can be varied, depending on the agent, or agents, and the desired effect. Thus, wherein the agent is genetic material, such as DNA, the process of administration involves administering a DNA encoding 2-5 AS, or a catalytically active fragment thereof, to a patient in need of such treatment. Administration can be accomplished by any means appropriate for the therapeutic agent, for example, by parenteral, mucosal, pulmonary, topical, catheter-based, or oral means of delivery. Parenteral delivery can include for example, subcutaneous intravenous, intrauscular, intra-arterial, and injection into the tissue of an organ, particularly tumor tissue. Mucosal delivery can include, for example, intranasal delivery. According to the method of the present invention, a nucleotide sequence encoding the 2-5 AS or catalytically active fragment is preferably administered into the airways of a patient, *i.e.*, nose, sinus, throat, lung, for example, as nose drops, by nebulization, vaporization, or other methods known in the art. Oral or intranasal delivery can include the administration of a propellant. Pulmonary delivery can include inhalation of the agent. Catheter-based delivery can include delivery by iontropheretic catheter-based delivery. Oral delivery can include delivery of a coated pill, or administration of a liquid by mouth. Administration can generally also include delivery with a pharmaceutically acceptable carrier, such as, for example, a buffer, a polypeptide, a peptide, a polysaccharide conjugate, a liposome, and/or a lipid. Gene therapy protocol is also considered an administration in which the therapeutic agent is a polynucleotide capable of accomplishing a therapeutic goal when expressed as a transcript or a polypeptide into the patient. Further information concerning applicable gene therapy protocols is provided in the examples disclosed herein.

The pharmaceutical compositions of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* (Martin EW [1995] Easton Pennsylavania, Mack Publishing Company, 19th ed.) describes formulations which can be used in connection with the subject invention. Formulations suitable for parenteral administration include, for example, aqueous sterile injection solutions, which may contain antioxidants, buffers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only

the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powder, granules, tablets, *etc.* It should be understood that in addition to the ingredients particularly mentioned above, the formulations of the subject invention can include other agents conventional in the art having regard to the type of formulation in question.

Therapeutically effective and optimal dosage ranges for 2-5 AS or catalytically active fragments thereof can be determined using methods known in the art. Guidance as to appropriate dosages to achieve an anti-viral effect is provided from the exemplified assays disclosed herein.

As used herein, the term “catalytic activity” with respect to fragments, analogues, and homologs of the 2-5 AS polypeptide, or to fragments, analogues, and homologues of nucleotide sequences encoding the 2-5 AS polypeptide, refers to 2’-5’ oligoadenylate synthetase activity. As used herein, “2’-5’ oligoadenylate synthetase activity” refers to polymerization of ATP to produce 2’-5’ linked oligoadenylates, which in turn, activate a latent ribonuclease, RNase L, that degrades RNAs (see, for example, Katze *et al.*, *Nat. Rev. Immunol.*, September 2002, 2(9):675-687; Justesen *et al.*, *Cell Mol. Life Sci.*, 57:1593-1612, 2000; Hartmann *et al.*, *J. Bio. Chem.*, 273(6):3236-3246, 1998; U.S. Patent No. 5,766,864). Preferably, the catalytic activity is an amount effective to inhibit RNA virus infection (pre-infection or post-infection). 2'-5' oligoadenylate synthetase activity can be determined directly or indirectly *in vivo*, or *in vitro*, using methods known in the art. Thus, cell-based assays can be utilized to determine whether an agent, such as a nucleotide sequence or polypeptide, exhibits the relevant catalytic activity, and can be utilized to carry out the method of RNA virus inhibition of the subject invention.

RNA virus infections that can be inhibited using the present invention include those that must produce double-stranded RNA as an intermediate step in viral replication and those viruses for which interferon can actively inhibit viral replication. These RNA viruses can included single-stranded or double-stranded RNA viruses, and have genomes of positive (+) or negative (-) strand polarity.

The present invention further provides methods of making the host cells, pharmaceutical compositions, and vectors described herein by combining the various components using methods known in the art.

The term “patient”, as used herein, refers to any vertebrate species. Preferably, the patient is of a mammalian species. Mammalian species which benefit from the disclosed

methods of treatment include, and are not limited to, apes, chimpanzees, orangutans, humans, monkeys; domesticated animals (*e.g.*, pets) such as dogs, cats, guinea pigs, hamsters, Vietnamese pot-bellied pigs, rabbits, and ferrets; domesticated farm animals such as cows, buffalo, bison, horses, donkey, swine, sheep, and goats; exotic animals typically found in zoos, such as bear, lions, tigers, panthers, elephants, hippopotamus, rhinoceros, giraffes, antelopes, sloth, gazelles, zebras, wildebeests, prairie dogs, koala bears, kangaroo, opossums, raccoons, pandas, hyena, seals, sea lions, elephant seals, otters, porpoises, dolphins, and whales. Human or non-human animal patients can range in age from neonates to elderly. The nucleotide sequences and polypeptides can be administered to patients of the same species or from different species. For example, mammalian, homologs can be administered to human patients.

The terms "2-5 AS p40 subunit" and "2-5 AS p40 subunit polypeptide" are used herein interchangeably to refer to the 2'-5' oligoadenlate synthetase p40 subunit gene or its coding sequence (CDS), its polypeptide product, or a catalytically active fragment or analogue of the polypeptide product, and includes 2-5 AS p40 subunit peptide homologs (such as mammalian orthologs (*e.g.*, SEQ ID NOs: 11 and 12); NCBI Accession Number M33863) and isoforms, unless otherwise noted. Thus, the term includes all splice variants of the p40 subunit, such as the 40kDa (SEQ ID NOs: 1 and 2), 42 kDa, and 46kDa (SEQ ID NOs:3 and 4) splice variants of the 2-5 AS p40 subunit (NCBI Accession Number NM_016816).

The terms "2-5 AS p69 subunit" and "2-5 AS p69 subunit polypeptide" are used herein interchangeably to refer to the 2'-5' oligoadenlate synthetase p69 subunit gene or its coding sequence (CDS), its polypeptide product, or a catalytically active fragment or analogue of the polypeptide product, and includes 2-5 AS p69 subunit peptide homologs (such as mammalian orthologs) and isoforms, unless otherwise noted. Thus, the term includes all splice variants of the p69 subunit, such as the 69kDa (SEQ ID NOs:5 and 6) and 71kDa (SEQ ID NOs:7 and 8) splice variants of the 2-5 AS p69 subunit (NCBI Accession Number NM_002535).

The terms "2-5 AS p100 subunit" and "2-5 AS p100 subunit polypeptide" are used herein interchangeably to refer to the 2'-5' oligoadenlate synthetase p100 subunit gene or its coding sequence (CDS) (SEQ ID NO:9), its polypeptide product (SEQ ID NO:10), or a catalytically active fragment or analogue of the polypeptide product, and includes 2-5 AS p100 subunit peptide homologs (such as mammalian orthologs) and isoforms, unless

otherwise noted. Thus, the term includes all splice variants of the p100 subunit (NCBI Accession Number AF063613).

The terms "comprising", "consisting of", and "consisting essentially of" are defined according to their standard meaning and may be substituted for one another throughout the instant application in order to attach the specific meaning associated with each term.

Materials and Methods

Epithelial Cell Culture, Virus Infection and Plaque Assay. The HEp-2 (ATCC CCL-23) cell line was obtained from the American Type Culture Collection (Manassass, VA) and was maintained in Minimum Essential medium with Hank's salts (MEM) supplemented with 5% fetal bovine serum (FBS) (ATLANTA BIOLOGICALS, Norcross, GA) at 37°C with 5% CO2. Respiratory syncytial virus (RSV) A2 strain was obtained from ATCC (VR-1302) and was propagated in HEp-2 cells grown in MEM with 2% FBS on a monolayer culture. Viral stocks were prepared from infected HEp-2 cells 5 days post infection (p.i.), stored at -700C in aliquots and used as the viral inoculum. RSV titers were quantified by plaque assay as described earlier (21).

MTT Cytotoxicity Assay. The effect of IFN-γ on the viability of cells was determined using a MTT [3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide] (SIGMA, St. Louis, MO) cytotoxicity assay. Triplicate sets of cell monolayers were used for each IFN-γ dose tested and for each time point. In this system, the mitochondrial dehydrogenase enzymes of living cells cleave the tetrazolium ring of the yellow MTT to form purple formazan crystals, which are insoluble in aqueous solutions. The crystals were dissolved in acidified isopropanol, and the absorbance of the resulting purple solution was spectrophotometrically measured at 540 nm. An increase or decrease in the viable cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the indicated dose of IFN-γ.

Immunoblot Analysis. IFN-γ treated cells were washed in cold PBS, pH 7.4 and scraped into PBS at various time points. The cells were collected by centrifugation at 6000 rpm for 3 min at 4°C and the cell pellet was suspended in a 2-pack volume of cell lysis buffer (50mM Tris-HCl, pH7.4; 1% NP-40; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1mg/ml aprotinin, leupeptin, pepstatin) and vortexed thoroughly. The cell lysate was spun at 13,000 rpm for 15' at 4oC to remove cellular debris. The supernatant was collected and the protein content estimated using the BCA (bicinchoninic acid) assay (PIERCE, Rockford, IL). 30mg

of total protein was mixed with an equal volume of 2X SDS sample buffer (22) and loaded onto a 10% SDS-PAGE and run at a 30mA constant current for 2 to 2.5 hours. For the detection of iNOS, the lysate of the IFN-γ and LPS- stimulated murine macrophage (RAW 264.7) was loaded onto the gel as a positive control. The proteins were transferred to a nitrocellulose membrane overnight at a 12mA constant current in transfer buffer (39mM glycine, 48mM Tris-HCl, 20% methanol) at 4°C.

Following protein transfer to the nitrocellulose membrane, the blots were immediately placed into blocking buffer (5% non-fat dry milk, 10mM Tris-HCl, pH 7.5, 100mM NaCl, 0.1% Tween 20) and incubated for 30' at room temperature. The blots were then individually incubated overnight with mAbs to IRF-1, IRF-2, PKR, cytokeratin-18 (SANTA CRUZ BIOTECHNOLOGY Inc, Santa Cruz, CA), iNOS (TRANSDUCTION LABORATORIES, Lexington, KY) and phospho-eIF-2a (CELL SIGNALING, Beverly, MA) at 4°C. Blots were washed three times in washing buffer (10mM Tris-HCl, pH 7.5, 100mM NaCl, 0.1% Tween 20) and were subsequently incubated with anti-mouse IgG HRP conjugate (BOEHRINGER MANNHEIM, Indianapolis, IN) (1:5000) for 30' at room temperature. The blots were again washed in washing buffer and developed by the addition of ECL chemiluminescent detection reagents (0.125 ml/cm2) according to the manufacturer's instructions (AMERSHAM LIFE SCIENCES, Arlington Heights, IL). The blots were wrapped in saran wrap and exposed to Kodak X-OMAT AR films (EASTMAN KODAK, Rochester, NY).

Nitrite Assay. Nitrite, a stable breakdown product of NO in physiological systems, was assayed using the Griess reaction (23). Cell culture supernatants (100 μL) were added in triplicates to 100 μL of Griess reagent (sulfanilamide 1%, naphthylethylenediamine dihydrochloride 0.1%, phosphoric acid 2.5%) using 96-well plates (SIGMA, St. Louis, MO). After incubation at room temperature for 10 min, absorbance at 550 nm was measured. A doubling dilution of a 50 μM sodium nitrite solution was used to generate a standard curve. The lower limit of the standard curve was 0.25 μM.

Northern Analysis. Northern blot analysis was performed to examine the mRNA expression profile of IFN-γ-induced genes. Total cellular RNA was isolated from cells using TRIZOL reagent (Life Technologies, Gaithersburg, MD) following the manufacturer's instructions. Probes for northern hybridization were prepared by RT-PCR using gene specific primers for IRF-1 (nucleotides 7-359), 2-5 AS p40 (nucleotides 2-492), 2-5 AS p69 (nucleotides 21-503), RSV G (nucleotides 4688-5584), RSV F (nucleotides 5661-7385) and glyceraldehyde 3 phosphate dehydrogenase (GAPDH) (nucleotides 1-360). The PCR

products were confirmed by sequencing. The probes were labeled using BrightStar Psoralen-Biotin labeling kit (AMBION, Austin, TX) following manufacturer's protocol. 10 mg of total RNA was size fractionated on 1% formaldehyde agarose gel, and transferred to nylon membranes (HYBOND N+, AMERSHAM, Piscataway, NJ) using standard protocol (24) and cross-linked by UV irradiation (UV STRATALINKER 1800, STRATAGENE, San Diego, CA). Hybridization was carried out at 420C overnight with 2-4 pM labeled probe and UltraHyb hybridization solution (AMBION, Austin, TX).

The blots were washed twice with 2x SSC, 0.1% SDS for 5 minutes each and two more washes with 0.1x SSC, 0.1% SDS for 15 minutes each at 420C. The blots were processed for detection using the BRIGHTSTAR BIODETECT Kit (AMBION, Austin, TX) following manufacturer's protocol. The blots were exposed to KODAK X-OMAT AR films (EASTMAN KODAK, Rochester, NY) for 1-15 minutes. The bands were quantified by using Advanced Quantifier software (BIOIMAGE, Ann Arbor, MI) and the signals were normalized with the respective GAPDH signal.

Antisense Blocking of 2'-5' Oligoadenylate Synthetase. Phosphorothioate antisense oligonucleotides (ODNs) were designed against p40 and p69 subunits of 2'-5' oligoadenylate synthetase. The sequences of antisense ODNs are as follows: p40 subunit, 5'-TTT CTG AGA TCC ATC ATT GA-3' (SEQ ID NO: 17) and p69 subunit, 5'- TCC CCA TTT CCC ATT GC-3'(SEQ ID NO: 18). The control ODN sequences 5'-GTC TAT GAA TAC TTT CCT AG-3' (SEQ ID NO: 19) and 5'-CAC CTC TAT CTC TCT CG-3' (SEQ ID NO: 20) are a scramble of the antisense sequence to p40 and p69 isomers, respectively, *i.e.*, identical in base composition. HEp-2 cells were treated with 1000 U/ml of IFN-γ protein for 20 hours. At the same time equimolar mixture of antisense ODNs to both the isoforms of 2-5 AS or their scrambled mismatch ODNs were added at concentrations 0, 3, 30 and 90 nM. Cells were infected with RSV at 20h post-IFN-γ-treatment, as described earlier. After 1h of virus adsorption, cultures were supplemented with complete medium, which contained 1000 U/ml of IFN-γ and respective concentrations of ODNs, and incubated for 72 hrs. ODNs were supplemented every 8 hours. At 72h pi, cells were washed three times with cold PBS, pH 7.4, harvested and the clear cell homogenate was used for plaque assay.

2-5 AS Assay. A 2-5 Assay was done following the method described previously (Ghosh *et al., J. Biol. Chem.* 272:15452-15458, 1997). Briefly, 20 μl of reaction mixture containing the cell homogenate, 20 mM Tris-HCl, pH 7.5, 20 mM magnesium acetate, 2.5 mM dithiothreitol, 5 mM ATP, 5 μCi of [a-32P]ATP, and 50 μg/ml poly(I)·poly(C) was

incubated for 3 h at 30 °C. The reaction was stopped by boiling for 3 min and centrifuged, and was incubated for 3 h at 37 °C with 3 µl of 1 unit/µl calf intestine alkaline phosphatase to convert the unreacted [a-32P]ATP to inorganic phosphate. Two µl of the sample were then spotted on a polyethyleneimine-cellulose thin layer chromatography plate and resolved in 750 mM KH2PO4, pH 3.5. The 2-5A formed was then quantified by using Advanced Quantifier software (BIOIMAGE, Ann Arbor, MI) and expressed as arbitrary units.

Generation of Stable Cell Line Overexpressing Rnase L Inhibitor. Human RLI cDNA was amplified as KpnI-BamHI cassette and cloned in pcDNA3.1 (INVITROGEN, Carlsbad, CA) by the standard procedure (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, and ed., Cold Spring Harbor Laboratory, NY, 1989). HEp-2 cells were transfected with 5mg of pcDNA3.1-RLI using lipofectine (LIFE TECHNOLOGIES, Gaithersburg, MD). The empty pcDNA3.1 vector was used as a control. Stable transfectants were selected by culturing the cells in the presence of G418 (LIFE TECHNOLOGIES, Gaithersburg, MD). Individual clones were isolated and analyzed for the expression of RLI mRNA. The clone that expressed RLI at the highest level and had a normal morphology and growth pattern was selected and named RLI-14.

RNAse L Assay. An RNAse L assay was done by ribosomal RNA cleavage assay (Player *et al.*, *Methods*, 15:243-253, 1998). Briefly, cells were harvested in NP-40 lysis buffer (10 mM HEPES, pH 7.5, 90 mM KCl, 1 mM magnesium acetate, 0.5% (v/v) NP-40, 2 mM 2-mercaptoethanol, 100 mg/ml leupeptin) and S10 lysate was prepared and protein content was estimated using the BCA (bicinchoninic acid) assay (Pierce, Rockford, IL). Ribosomal RNA cleavage by RNAse L was assayed in a 20 ml reaction containing 200 mg protein, 2 ml of 10x cleavage buffer (100 mM HEPES, pH 7.5, 1 mM KCl, 50 mM magnesium acetate, 10 mM ATP, 0.14 M 2-mercaptoethanol), 100 nM 2'-5'A and incubated at 300C for 2h. RNA was isolated from the reaction using TRIZOL reagent (LIFE TECHNOLOGIES, Gaithersburg, MD) following the manufacturer's instructions. 2 mg of RNA was separated on agarose gel electrophoresis and the rRNA cleavage products were compared.

Animals. Female 6-8 weeks old wild type and STAT4$^{-/-}$ BALB/c mice from Jackson Laboratory (Bar Harbor, ME) were maintained in pathogen free conditions at the animal center at USF College of Medicine. All procedures were reviewed and approved by the committee on animal research at the University of South Florida College of Medicine.

<u>Cloning and recombination of adenoviral vectors</u>. Murine 25AS(p40) cDNA was cloned into adenoviral transfer vector pShuttle-CMV (STRATAGENE, CA) at KpnI and XhoI sites. The left and right arms of pShuttle-CMV vector contains Ad5 nucleotides 34,931-35,935 and 3,534-5,790, which mediate homologous recombination with pAdEasy-1 vector in E. coli, plus inverted terminal repeat (ITR) and packaging signal sequences (nucleotides 1-480 of Ad5) required for viral production in mammalian cells. pAdEasy-1 adenoviral plasmid (STRATAGENE, CA) contains all Ad5 sequences except nucleotides 1-3,533 (encompassing the E1 gene) and nucleotides 28,130-30,820 (encompassing E3).

For generation of recombinant adenovirus plasmid, pShuttle-CMV-p40/LacZ plasmids were linearized with PmeI, and co-transformed with pAdEasy-1 plasmid into recombination proficient BJ5183 cells. The recombination was confirmed by PacI digestion. The recombined clones were re-transformed into DH5α cells for large-scale plasmid purification.

<u>Generation and purification of recombinant adenovirus</u>. HEK293 cells, which produce the deleted E1 genes in trans, were transfected with 4 μg of PacI digested recombinant adenovirus plasmid DNA with lipofectin (LIFE TECHNOLOGIES, MD). Cells were harvested 7-10 days post-transfection, resuspended in PBS and recombinant virus was collected by 3-4 freeze-thaw cycles. The recombinant virus expressing murine p40 and LacZ were termed Ad-p40 and Ad-LacZ, respectively. The viruses were amplified by infecting fresh HEK-293 cells. Viruses were further purified by CsCl banding (Becker *et al.*, *Methods Cell Biol.*, 43 Pt. A:161-189, 1994). The viral band was extracted and CsCl was removed by passing through Centricon-100 columns (MILLIPORE, MA).

<u>Quantitation of RSV titers in lung</u>. To quantify RSV titers in the mouse lung whole lungs were first weighed and placed immediately in EMEM media supplemented with 10% FBS. Lungs were homogenized, centrifuged at 10,000 RPM for 10 minutes at 4° C, the clear supernatants were used for plaque assays by shell vial technique (Kumar et al., 2002).

<u>Pulmonary Function</u>. To evaluate the pulmonary function in vaccinated and control groups, mice were administered IGT, as described earlier. Three days later, airway responsiveness was assessed non-invasively in conscious, unrestrained mice with a whole body plethysmograph (BUXCO ELECTRONICS, Troy, NY), as previously described (Matsuse *et al.*, *J. Immunol.* 164:6583-6592, 2000). With this system, the volume changes that occur during a normal respiratory cycle are recorded as the pressure difference between an animal-containing chamber and a reference chamber. The resulting signal is used to

calculate respiratory frequency, minute volume, tidal volume, and enhanced pause (Penh). Penh was used as the measure of bronchoconstriction and was calculated from the formula: Penh = pause × (peak expiratory pressure/peak inspiratory pressure), where pause is the ratio of time required to exhale the last 30% of tidal volume relative to the total time of expiration. Mice were placed in the plethysmograph and the chamber was equilibrated for 10 min. They were exposed to aerosolized PBS (to establish baseline) followed by incremental doses (6, 12.5, 25, and 50 mg/ml) of methacholine (SIGMA CHEMICALS, St. Louis, MO). Each dose of methacholine was aerosolized for 5 min, and respiratory measurements were recorded for 5 min afterward. During the recording period, an average of each variable was derived from every 30 breaths (or 30 s, whichever occurred first). The maximum Penh value after each dose as used to measure the extent of bronchoconstriction.

Bronchoalveolar lavage (BAL) and histology of the lung. Bronchoalveolar lavage were performed on Ad-p40 administered and control mice, as described before (Kumar *et al.*, 1999). Histological staining and a semiquantitative analysis of airway inflammation from the lungs of p40 treated and control groups of mice were performed, as described earlier (Kumar *et al.*, 1999). Lung inflammation was assessed after staining the sections with hematoxylin and eosin (HE). The entire lung section was reviewed, and pathological changes were evaluated for epithelial damage, peribronchovascular cell infiltrate, and interstitial-alveolar cell infiltrate for the mononuclear cells and polmorphonuclear cells.

Statistical Analysis. Experiments were repeated 2 to 4 times for each experiment as indicated. Statistical significance was analyzed using paired two-tailed student's t-test. Differences were considered statistically significant when the p-value was less than 0.05.

Example 1: IFN-γ Attenuates RSV Infection in Human Epithelial Cells

To examine the effect of IFN-γ on RSV infection, HEp-2 cells were pre-incubated for 4-20 h with different concentrations of IFN-γ and subsequently infected with RSV. Respective concentrations of IFN-γ were added back to the cells in complete medium after the removal of viral inoculum. Cells were harvested at 72h p.i., and viral titer was determined by plaque assay. RSV replication was inhibited significantly with the addition of various concentrations of IFN-γ to both cell lines prior to RSV infection (Figures 1A and B). A 97% inhibition of replication was observed in HEp-2 and A549 cells, at 1000 U/ml of IFN-γ added at 20h pre-infection. Cells treated with IFN-γ 4h pre-infection also showed significant reduction ($p<0.01$) in RSV titer (50% reduction). A significant decrease ($p<0.01$)

in RSV titer (39% reduction) was observed in A549 cells, which were not treated with IFN-γ before infection, but were only treated at 1h post infection (Figure 1C). To rule out the possibility that the reduction in RSV titers could be due to cytotoxicity of IFN-γ, a MTT cytotoxicity assay was performed. The results indicate that the cells were metabolically as viable as the untreated control cells when treated with the highest concentrations of IFN-γ (1,000 U/ml; Figure 1 D). Thus, IFN-γ did not exhibit any cytotoxic or growth inhibitory effect on these cells. These results suggest that the treatment of cells with soluble IFN-γ results in a significant decrease in RSV infection in epithelial cells.

Example 2: IFN- γ Induces IRF-1 Protein Expression

ISGs implicated in the antiviral activity of IFNs include IRFs, double stranded RNA activated protein kinase (PKR) and inducible nitric oxide synthase (iNOS). To identify the ISGs in these cells potentially involved in protection against RSV infection, proteins were analyzed from cells at various time points post treatment with IFN-γ (1000 U/ml). A western blot analysis was performed using specific antibodies to IRF-1, IRF-2 and PKR (Figure 2A). There was increased expression of IRF-1 but no change in the expression of IRF-2 following IFN-γ addition. Expression of IRF-1 increased after 30' of IFN-γ addition. The expression of PKR decreased gradually over time (Figure 2B) and no change in the expression of phospho-eIF-2a was observed following IFN-γ addition. Cytokeratin-18 was used as an internal control, the expression of which did not change with the addition of IFN-γ. To examine if IFN-γ induced iNOS plays a role in antiviral action, iNOS expression was examined by western blotting (Figure 2B). The expression of iNOS protein could not be detected before and after IFN-γ addition. Murine macrophage cell lysate containing iNOS was used as a positive control, which did not bind to the cytokeratin-18 antibody used as internal control. To rule out completely the involvement of iNOS in the antiviral effect of IFN-γ, the level of nitric oxide (NO) was examined in the culture supernatant of both HEp-2 and A549 cells before and after the addition of IFN-γ at various time points. No detectable level of NO (lowest concentration of standard was 0.25 mM) was observed in both cell lines at any time point, *i.e.*, before or after IFN-γ addition in both cell lines. A similar expression pattern was observed for IRF1, IRF2, PKR and iNOS in A549 cells. These results indicate that IFN-γ up-regulates IRF-1 in these cells and neither PKR nor iNOS play any role in the antiviral activity of IFN-γ against RSV infection in human epithelial cell lines.

Example 3: Exogenous IFN-γ Upregulates mRNA Synthesis Of IRF-1 and 2-5 AS

IRF-1 has been reported to play a role in antiviral activity via the induction and activation of 2-5 AS (Reis *et al., EMBO J.* 11:185-193, 1992). Northern analysis was performed using gene specific probes for IRF-1 and the p40 and p69 isoforms of 2-5 AS (Figure 3). The IRF-1 mRNA was induced at 30 min after addition of IFN-γ and continued to increase gradually thereafter until 48h post exposure. The induction of the p40 and p69 isoforms of 2-5 AS was observed starting at 4h and peaked at 24h post exposure. The p40 probe hybridized to two transcripts of 1.8 and 1.6 kbp. Similarly, the p69 probe hybridized to four expected transcripts of 5.7, 4.5, 3.7 and 3.2 kbp of which 5.7 kbp was the major transcript. These results suggest that IFN-γ induces IRF-1, which in turn, up regulates 2-5 AS, suggesting that the latter may be involved in the anti-RSV mechanism of IFN-γ.

Example 4: 2-5 As Antisense Oligonucleotides Abrogate the Antiviral Effect Of IFN-γ in HEp-2 Cells

To investigate whether IFN-γ induced anti-RSV activity is mediated by 2-5 AS, IFN-γ exposed (1000 U/ml at 20h pre-infection) HEp-2 cells were treated with equimolar mixture of antisense oligonucleotides (ODNs) to both p40 and p69 isoforms of 2-5 AS. Scrambled mismatch of the antisense ODN sequence to p40 and p69 at the same concentration were used as control. RSV infection was barely detectable in cells either treated with IFN-γ alone or with cells treated with IFN-γ and control ODNs but not in those treated with IFN-γ and antisense ODNs, as shown in Figure 4. Addition of antisense ODN significantly reverted ($p< 0.01$) the antiviral effect of IFN-γ against RSV infection and this reversal was dose-dependent and increased with increasing concentrations of antisense ODNs. As shown in Figure 4, 2-5 AS activity was reduced in a dose dependent manner in the cells treated with antisense ODN to 2-5 AS but not control ODN. These results indicate that the addition of antisense ODNs to 2-5 AS to IFN-γ-treated cells reduced 2-5 AS activity in these cells and in turn the antiviral effect of IFN-γ.

Example 5: Overexpression Of Rnase L Inhibitor (RLI) Does Not Alter The IFN-γ Responses In Hep-2 Cells

In addition to RNAse L, RNAse L inhibitor (RLI) has been implicated in the antiviral effect of IFN-γ. To determine the role of 2-5A/RNase L-mediated antiviral mechanism, a

stable cell line expressing RLI, RLI-14, was established. A northern analysis of RNAs from RLI-14 and HEp-2 using gene specific probe for RLI showed a major 3.5kb transcript and a minor 2.8 Kb transcript (Figures 5A and 5B). A seven-fold increase in the major RLI transcript expression was observed in RLI-14 cells when compared to HEp-2 cells. The analysis of IFN-γ induced proteins in RLI-14 cell line by western blotting showed that IFN-γ induced expression of IRF-1, but not IRF-2, at 30 min post induction and IRF-1 expression continued to increase thereafter until 48h (Figure 5C) as in HEp-2 cells (Figure 2A). Also, a time-specific decrease in PKR protein concentration was observed after IFN-γ addition in the RLI-14 cell line. The expression of cytokeratin-18, used as an internal control, remained unchanged with IFN-γ addition. The level of mRNA expression of IRF-1, p40 and p69 isoforms of 2-5 AS was observed by northern analysis, and the expression level showed a gradual increase over time following IFN-γ stimulation (Figure 5D) as in HEp-2 cells (Figure 3). These results suggest that overexpression of RLI does not change the expression pattern of the IFN-γ-induced genes involved in antiviral activity of these cells.

Example 6: Rnase L Inhibitor (RLI) Overexpression Decreases The Antiviral Activity Of IFN- γ

To examine the effect of the overexpression of the RNase L inhibitor, both HEp-2 and RLI-14 cells were treated with IFN-γ at 100-1000 U/ml at 20h pre-infection and subsequently infected with RSV. IFN-γ was added back to the cells at respective concentrations following RSV infection. HEp-2 cells treated with 100 and 1000 U/ml of IFN-γ showed significant inhibition ($p< 0.001$) of RSV infection (72% and 97% reduction, respectively) when compared to untreated cells. In marked contrast, RLI-14 cells showed significantly lower inhibition of infection (only 12% and 22% reduction, respectively) compared to HEp-2 cells at respective concentrations of IFN-γ, as showed in Figure 6. In absence of IFN-γ treatment, both cell lines exhibited identical RSV titers upon infection. However, the viral titer significantly decreased ($p< 0.01$) when the concentration of IFN-γ was increased from 100 U/ml to 1000 U/ml in RLI-14 cells. This demonstrates that increase in IFN-γ led to higher expression of 2-5 AS and in turn production of 2-5A, which subsequently bound to RNase L and increased the level of active RNase L by releasing RNase L from its inactive complex. Reduction in virus replication was inhibited in RLI-14 cells (%) when compared to HEp-2 cells (%), as shown in Figure 6. In order to examine whether the reduction in inhibition of RSV infection in RLI-14 cells was due to reduced RNAse L activity in these cells, RNAse L

assay was done using ribosomal RNA cleavage assay. This reaction uses cell lysate as a source of both substrate and enzyme, thus giving a comparison of the ribonuclease activity of RNAse L in different cell types. The results confirm that ribonuclease activity of RNAse L is indeed reduced in RLI-14 cells when compared to HEp-2 cells as evident from the rRNA cleavage products, as shown in Figure 6. Together, these results confirm the involvement of 2-5A/RNase L in the antiviral effect of IFN-γ against RSV infection.

The finding that treatment of HEp-2 and A549 cells at 20h pre-infection with as low as 100 U/ml of IFN-γ proteins inhibits RSV infection and replication when compared to untreated cells, has significant therapeutic implications. HEp-2 and A549 cells treated with 1000 U/ml of IFN-γ at 20h pre-infection exhibited a 97% (30-31 fold in log10 PFU/ml) reduction in RSV titer. The RSV titer also decreased by 39% (1.7 fold reduction in log10 PFU/ml) in these cells, which were not treated with IFN-γ prior to infection but were only treated immediately after RSV infection. RSV is resistant to the antiviral effects of type-I interferons and human MxA. It has been reported that overexpression of IFN-γ by gene transfer and by recombinant RSV attenuates RSV replication in a mouse model of RSV infection. However, the mechanism of antiviral action of IFN-γ against RSV is not known.

The elucidation of the mechanism underlying IFN-γ-mediated resistance to RSV infection in human epithelial cells has been the main focus of this invention. The mechanism of antiviral action of IFN-γ is complex and may be unique for individual cell lines and viruses. A profile of ISGs, relevant to antiviral activity in these epithelial cells, establish that IFN-γ exposure results in induction of both the mRNA and protein for IRF-1 but not IRF2. In non-induced cells the IRF-2 protein functions as a repressor of ISGs. IFN-γ induction temporarily removes this repression and activates ISGs including IRF-1. IRF-1 and IRF-2 compete for the same cis acting recognition sequences but with opposite effects. Findings in these epithelial cells are consistent with those found for human macrophages, where IFN-γ treatment does not enhance IRF-2 gene expression, despite strong upregulation of IRF-1 mRNA expression. Two additional ISGs, PKR and iNOS proteins were examined for their role in IFN-γ induced antiviral activity. IFN-γ activates PKR, which in turn phosphorylates and inactivates eukaryotic initiation factor-2a (eIF-2a) and leads to restriction of cellular as well as viral protein synthesis. The iNOS is also known to mediate antiviral property of IFN-γ. However, a time specific decrease in PKR expression and no change in phosphorylation of eIF-2a and the lack of detectable levels of iNOS protein or NO in IFN-γ-stimulated HEp-2

and A549 cells indicate that neither PKR and phospho-eIF-2a nor iNOS play any role in IFN-γ mediated inhibition of RSV infection in these cells.

To further dissect the mechanism of IFN-γ mediated anti-RSV activity in HEp-2 and A549 cells, IRF-1 induced expression of 2-5 AS was examined. Of the four isoforms (p40, p46, p69, and p100) of 2-5 AS detected in human cells to date, the expression pattern of the p40 and p69 isoforms following IFN-γ stimulation was examined in this study because of the following. The p40 and p46 isoforms of 2-5 AS, which are dependent on dsRNA for activation, are derived from the same gene by differential splicing between the fifth and an additional sixth exon of this gene and are thus identical for the first 346 residues, except for their C-terminal ends. Of the two high molecular weight isoforms, p69, but not p100, requires dsRNA for activation. The expression of 2-5 AS p40 and p69 are induced by IFN-γ in these cells at 4h and peaks at 24h post IFN-γ addition. Therefore, the antiviral effect of IFN-γ in these cells is observed when the cells are treated with IFN-γ at 4h pre-infection and is highest when treated at 20h pre-infection as the level of 2-5 AS is at peak at that time. These data suggest that the antiviral mechanism of IFN-γ against RSV infection is mediated by the activation of IRF-1, which in turn activates the 2-5 AS system. A dose-dependent abrogation of 2'-5' AS activity and in turn the anti-RSV effect of IFN-γ by the addition of an equimolar mixture of antisense ODNs to p40 and p69, but not by the scrambled mismatch ODNs, provide evidence supporting the role of 2-5 AS in the antiviral mechanism of IFN-γ against RSV infection.

2-5 AS induces 2-5A, which binds to and activate RNase L, which cleaves double stranded RNA 3' of UpN residues. The levels of RNase L are increased in growth-arrested cells and following IFN-γ treatment; however, its biological activity is thought to be controlled at the level of enzymatic activation rather than through regulation of its transcription and translation. Increasing endogenous levels of 2-5A leads to enhanced RNase L activity, which suggests that intracellular levels of 2-5A are rate limiting in the activation of RNase L, whereas cellular levels of RNase L are sufficient for maximal biological activity. Furthermore, RNase L remains in an inactive form in the cells being bound to an inhibitor, RLI, which codes for a 68kDa protein whose mRNA is not regulated by IFN-γ. RLI induces neither 2-5A degradation nor reversible modification of RNase L when expressed in a reticulocyte lysate, but antagonizes the binding of 2-5A to RNase L, thus, its nuclease activity, since 2-5A binding is a prerequisite to RNase L dimerization and activation.

RLI-14, a stable cell line overexpressing RLI, was established from HEp-2 cells and characterized to determine precisely the involvement of RNase L in the antiviral mechanism of RSV infected epithelial cells. The finding that RLI-14 was almost identical to the parent HEp-2 cells in its response to IFN-γ shows that RLI overexpression does not alter the induction of ISGs in these cells (Figures 5A-D). Nonetheless, reduced RNAse L activity and antiviral activity of IFN-γ in RLI-14 cells (Figure 6), confirmed that the RNase L activity is indeed critical to the antiviral effect of IFN-γ and is only partly controlled by the elevated levels of 2-5 AS in these cells following IFN-γ treatment. The reduction in antiviral effect of IFN-γ in these cells was dependent on the dose of IFN-γ, indicating that the level of 2-5A, which is regulated by IFN-γ and the level of RLI are crucial in determining which pathway cells will follow. The importance of the level of 2-5A was confirmed by preliminary findings which showed significant reduction in RSV infection when HEp-2 cells were treated with 100 U/ml of IFN-γ 20h pre-infection and transfected with 1mM 2-5A 2h pre-infection, when compared to the cells treated with 100 U/ml of IFN- g alone. Similarly the importance of the level of RLI in the antiviral activity was reported for HIV, where RLI is induced during HIV1 infection and down regulates the 2-5A/RNase L pathway in human T cells.

In summary, these results demonstrate that IFN-γ inhibits RSV infection of human epithelial cells. Specifically, in HEp-2 and A549 epithelial cells, IFN-γ upregulates IRF-1, which in turn, induces 2-5 AS. Further, the 2-5 AS generates 2-5A that activates RNase L, which is normally found in the cytoplasm in inactive state bound to RLI. Thus, RNase L-mediated cleavage of viral RNA is governed by the ying-yang mechanism involving 2-5A and RLI. In a 2-5A-dominant state cells are protected from RSV infection due to the activation of RNase L. In contrast, an RLI-dominant condition attenuates the antiviral effect by inactivation of RNase L. Since, 2-5A and RLI are respectively, governed by IFN-γ-dependent and independent mechanisms, treatment with IFN-γ or overexpression of 2-5 AS should provide an efficient means to redirect the 2-5A:RLI ratio toward a shift in favor of 2-5A and achieve a profound antiviral effect.

<u>Example 7: 2-5 AS plasmid DNA vaccination attenuates RSV infection and pathogenesis</u>

As shown in Figure 8A, 2-5 AS pDNA vaccine decreases lung RSV titers. BALB/c mice (n=4) were intranasally administered with p2'-5' AS (25 mg of DNA each time complexed with lipofectamine) or an equal amount of empty pVAX vector DNA 3 times in 2-day intervals. Mice were infected with RSV seven days after last DNA administration and

were sacrificed on day 5 post-infection. BAL was performed and lungs were collected. RSV titer was determined by plaque assay from the lung homogenate. The results show that 2-5 AS cDNA vaccination can significantly attenuate lung titers of RSV in infected mice.

Figure 8B shows that reduction of viral titers is associated with reduction in MIP-1α. Expression level of MIP-1α was determined from lung homogenate by ELISA. The results show that vaccination with 2-5 AS cDNA decreases production of chemokine MIP-1α which is known to be proinflammatory in action.

In Figure 8C, 2-5 AS overexpression increased the macrophage population significantly compared to RSV infected mice. BAL cell differential was performed and percentages of macrophage, lymphocytes, and neutrophils were determined. The results show that 2-5 AS does not alter the cellular composition of the lung. No significant changes are seen in lymphocytes and macrophages, however the percent of neutrophils is increased in the lungs of mice treated with 2-5 AS cDNA. Figures 9A-9C show that 2-5 AS vaccination significantly decreased pulmonary inflammation. Histological sections from lungs were stained with hematoxylin and eosin and representative photomicrographs are shown.

Example 8: Ad -2-5AS(p40) decreases lung RSV titers

A reduction in virus titer is the gold standard by which the effectiveness of an antiviral therapy is measured. Mice were intranasally administered with 10^8 PFU/ml rAD-p40 and then infected with RSV 4 h later. Analysis of lung virus titers following acute, live RSV infection at day 5 post infection show a significant (100-fold, $P<0.01$) reduction in RSV titers in Ad-p40 treated mice compared to controls (Figure 10). These results indicate that the rAD-p40 treatment constitutes an effective prophylaxis against RSV infection.

Example 9: Ad -2-5AS(p40) decreases AHR in mice

The safety of an antiviral therapy especially can be measured by a decrease in RSV – induced AHR. To test whether Ad-p40 administration reduces RSV-induced airway hyperreactivity, the % baseline enhanced pause (Penh) was measured in a group of mice treated with rAD-p40 prior to RSV infection and their AHR was compared with untreated RSV infected group. Mice receiving Ad-p40 exhibited a similar response to methacholine challenge when compared to uninfected PBS control group (Figure 10). These results suggest that the Ad-p40induced decrease in RSV infection decreases AHR.

Example 10: Ad -2-5AS(p40) normalizes cellular infiltration to the lung

The inflammation in the lung due to RSV infection is due infiltration into the lung a large number of macrophages and lymphocytes. To determine whether treatment with rAD-p40 affects migration of these cells to the lung, mice administered with rAD-p40 and RSV infected were compared to RSV infected mice without treatment and naïve mice as control and to rAd-lacZ as control. Mice with p40 gene transfer and RSV infection show a BAL cell differential similar to that of normal uninfected animals, lack of AHR compared to RSV-infected animals without p40 gene transfer and lack of the peribronchiolar and perivascular inflammation suggesting that intranasal p40 can potentially be an effective anti-viral approach *in vivo* for RSV infection.

Example 11: Ad -2-5AS(p40) decreases RSV infection-induced pulmonary inflammation

Lung inflammation was examined in different groups of mice. Mice treated with Ad-p40 and Ad-lacZ upon acute RSV infection exhibit relatively less disruption of the epithelium and cellular infiltration. Representative pathological features reveal that group of mice receiving Ad-p40 exhibit less epithelial damage, and reduced mononuclear cell (MNC) and polymorphonuclear cell (PMNC) infiltrates in the interstitial and peribronchovascular region, as compared to Ad-lacZcontrols (Figures 12A-12H). These results suggest that the Ad-p40 protects mice from RSV infection-induced pulmonary inflammation. These results suggest that Ad-p40 protects mice from RSV infection-induced pulmonary inflammation.

The finding that transient gene expression therapy can substantially reduce lung RSV titers by 2 logs (100–fold), inhibit RSV-infection induced AHR and make the lung resistant to inflammation by acute RSV infection is very significant. These results suggest tremendous therapeutic potential of this approach. The other members of this family include the measles virus, the sendai virus, parainfluenza 1, 2, and 3, the mumps virus, the simian virus, and the newcastle virus. This finding is also important for other Paramyxoviruses, such as rotavirus that causes juvenile diarrhea in millions of children worldwide. Furthermore, beyond this family of viruses, the 2-5 AS/RNase L cascade has been implicated in the anti-viral activity of picorna viruses, (Hassel, BA *et al. Embo J*, 1993, 12(8):3297-304; Benavente, J *et al. J Virol.* 1984, 51(3):866-71; Goswami, BB and Sharma, OK. *J Biol Chem*, 1984, 259(3):1371-4; Nilsen, TW *et al. Mol Cell Biol*, 1983, 3(1):64-9), vaccinia virus (Maitra, RK and Silverman, RH. *J Virol*, 1998, 72(2):1146-52; Banerjee, R *et al. Virology*, 1990, 179(1):410-5), reovirus (Kumar, R *et al. J Virol*, 1988, 62(9):3175-81), HIV (Saito, H *et al. Keio J Med*,

1996, 45(3):161-7; 45), EMCV (Glezen, WP *et al. Am J Dis Child*, 1986, 140(6):543-6), Hepatitis B and C virus (Groothuis, JR *et al. Pediatrics*, 1988, 82(2):199-203; Nelson WE, Behrman RE, Kliegman R. *Nelson Textbook of Pediatrics*. 15 ed. Philadelphia).

Moreover, besides human disease, this finding may have implications, for RSV of cattle (BRSV), sheep, and goats. If 2-5AS mediated approach is successful, the mortality and morbidity due to RSV infection can be reduced. Also, RSV has been linked with the development of asthma, and hence, prevention or successful treatment of RSV is expected to decrease the incidence of asthma and fatal exacerbation of asthma due to RSV. Adults infected with RSV miss work for an average of 7-9 days as opposed those with flu who miss an average of 6-7 days. Therapeutic treatment can reduce the number of absences from the work, which exceeds the flu infection. Also, prophylaxis prior to and during viral season and treatment immediately after infection can lead to a substantial decrease in hospitalization and emergency visits due to RSV infection.

RSV is one of the important viral respiratory pathogen that produces an annual epidemic of respiratory illness. In children, common diseases associated with RSV infection primarily include interstitial lung diseases, such as bronchiolitis, and asthma. RSV is estimated to cause 85% of the cases of acute bronchiolitis that affects infants and young children (Shay, DK *et al. Jama*, 1999, 282(15):1440-6). Some children may become infected during three or four successive RSV seasons. Each year, RSV is responsible for up to an estimated 125,000 pediatric hospitalizations, with a mortality rate of approximately 2% (Heilman, CA. *J Infect Dis* 1990, 161(3):402-6; Shay, DK *et al. J Infect Dis*, 2001, 183(1):16-22; Altman, CA *et al. Pediatr Cardiol*, 2000, 21(5):433-8; Simoes, EA. *Lancet*, 1999, 354(9181):847-52; Falsey, AR. *et al. J Infect Dis*, 1995, 172(2):389-94). Among hospitalized infants with chronic lung and heart disease, the RSV mortality rate may be as high as 5%. Up to half of all pediatric admissions for bronchiolitis and one-quarter of admissions for pneumonia are due to RSV (La Via *et al., Clin. Pediatr. (Phila)*, 32(8):450-454, 1993). RSV is a major risk factor for a number of other health conditions, such as immuno-deficiency, cardiac arrhythmia, congenital heart disease, and unusual atrial tachycardia (Donnerstein *et al., J. Pediatr.* 125(1):23-28, 1994).

Emerging evidence also suggests that RSV is an important pathogen in profusely healthy adults as well (Hall *et al., Clin. Infect. Dis.* 33(6):792-796, 2001). In a study of 15 adults who were challenged by RSV after a natural infection, 50% were reinfected after two months; by 26 months 73% were reinfected (Fixler, DE. *et al. Pediatr Cardiol*, 1996,

17(3):163-8). RSV infection is also clinically important in previously healthy working adults (Hogg, JC. *et al. American Journal of Respiratory & Critical Care Medicine*, 1999, 160(5):S26-S28). In this study, of a total of 2960 18-60 year-old adults studied during 1975 to 1995, 211 (7%) acquired RSV infection; 84% of infections were symptomatic –74% upper respiratory tract infection, 26% lower respiratory tract infection and 40% were febrile. RSV is a major risk factor for the development and/or exacerbation of asthma and chronic obstructive pulmonary disorder (COPD), and about 30 million of Americans suffers from asthma and COPD.

The prevalence of bronchiolitis in infants as well as asthma and COPD has increased throughout the world, including in the United States, over the past two decades. The rates of death from asthma have increased from 0.8 per 100,000 in 1977-78 to 2.0 per 100,000 in 1991, and these rates have increased in almost all age groups in the United States (Sly, RM. *Ann Allergy*, 1994, 73(3):259-68). Asthma is the most common cause of the admission of children to the hospital, and it is the most common chronic illness causing absence from school and work in North America (Nelson, RP, Jr., *et al. J Allergy Clin Immunol*, 1996, 98(2):258-63). The total costs of illnesses related to asthma in 1990 were 6.2 billion, a 53% increase in direct medical expenditures and a 23% increase in indirect costs since 1985 (Weiss, KB *et al. N Engl J Med*, 1992, 326(13):862-6). The total estimated cost in 1995 for the treatment of allergic diseases, asthma, chronic sinusitis, otitis media, and nasal polyps, was about $10 billion (Baraniuk, JN. *J Resp Dis*, 1996, 17(S11)). Together, these diseases lead to a significant reduction in the quality of life and a tremendous economic loss.

Finally, although studies with 2-5AS (p40) in the adenovirus system provides the "proof of concept" for the anti-RSV activity, other virus vectors, including adeno-associated vectors (Zhao, N *et al. Mol Biotechnol*, 2001, 19(3):229-37; Monahan, PE *et al. Mol Med Today*, 2000, 6(11):433-40; Senior, K. *Lancet*, 2002, 359(9313):1216) can be used to express this p-40 or other 2-5AS gene(s) for the antiviral activity.

<u>Example 12: Gene Therapy</u>

In the therapeutic and prophylactic methods of the present invention, the nucleotide sequence encoding 2-5 AS, or a catalytically active fragment thereof, can be administered to a patient in various ways. It should be noted that the vaccine can be administered as the compound or as pharmaceutically acceptable salt and can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, diluents,

adjuvants and vehicles. In those cases in which the RNA virus is a virus that infects the patient's respiratory system, the compounds can be administered intranasally, bronchially, via inhalation pathways, for example. The patient being treated is a warm-blooded animal and, in particular, mammals including man. The pharmaceutically acceptable carriers, diluents, adjuvants and vehicles as well as implant carriers generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating material not reacting with the active ingredients of the present invention.

It is noted that humans are treated generally longer than the mice exemplified herein, which treatment has a length proportional to the length of the disease process and drug effectiveness. The doses may be single doses or multiple doses over a period of several days, but single doses are preferred.

The carrier for gene therapy can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

Proper fluidity, when desired, can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Nonaqueous vehicles such a cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be used as solvent systems for compound compositions. Additionally, various additives that enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the compounds.

Examples of delivery systems useful in the present invention include, but are not limited to: U.S. Pat. Nos. 5,225,182; 5,169,383; 5,167,616; 4,959,217; 4,925,678; 4,487,603; 4,486,194; 4,447,233; 4,447,224; 4,439,196; and 4,475,196. Many other delivery systems and modules are well known to those skilled in the art.

A pharmacological formulation of the nucleotide sequence utilized in the present invention can be administered orally to the patient. Conventional methods such as administering the compounds in tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like are usable. Known techniques which deliver the vaccine orally or intravenously and retain the biological activity are preferred.

In one embodiment, the nucleotide sequence can be administered initially by nasal infection to increase the local levels of 2-5 AS enzymatic activity. The patient's 2-5 AS activity levels are then maintained by an oral dosage form, although other forms of administration, dependent upon the patient's condition and as indicated above, can be used. The quantity of vaccine to be administered will vary for the patient being treated and will vary from about 100 ng/kg of body weight to 100 mg/kg of body weight per day and preferably will be from 10 mg/kg to 10 mg/kg per day.

As indicated above, standard molecular biology techniques known in the art and not specifically described can be generally followed as in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (1989), and in Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989) and in Perbal, A Practical Guide to Molecular Cloning, John Wiley & Sons, New York (1988), and in Watson *et al.*, Recombinant DNA, Scientific American Books, New York and in Birren *et al.* (eds) Genome Analysis: A Laboratory Manual Series, Vols. 1-4 Cold Spring Harbor Laboratory Press, New York (1998) and methodology as set forth in U.S. Patent Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659; and 5,272,057, the contents of which are incorporated herein by reference in their entirety. Polymerase chain reaction (PCR) can be carried out generally as in PCR Protocols: A Guide To Methods And Applications, Academic Press, San Diego, Calif. (1990). *In-situ* (In-cell) PCR in combination with Flow Cytometry can be used for detection of cells containing specific DNA and mRNA sequences (Testoni *et al.*, 1996, *Blood* 87:3822).

As used herein, the term "gene therapy" refers to the transfer of genetic material (*e.g.*, DNA or RNA) of interest into a host to treat or prevent a genetic or acquired disease or condition phenotype. The genetic material of interest encodes a product (*e.g.*, a protein, polypeptide, peptide or functional RNA) whose production *in vivo* is desired. For example, in addition to the nucleotide encoding 2-5 AS, or a catalytically active fragment thereof, the genetic material of interest can encode a hormone, receptor, or other enzyme, polypeptide or

peptide of therapeutic value. For a review see, in general, the text "Gene Therapy" (*Advances in Pharmacology* 40, Academic Press, 1997).

Two basic approaches to gene therapy have evolved: (1) *ex vivo* and (2) *in vivo* gene therapy. In *ex vivo* gene therapy, cells are removed from a patient, and while being cultured are treated *in vitro*. Generally, a functional replacement gene is introduced into the cell via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination, *etc.*) and an expression system as needed and then the genetically modified cells are expanded in culture and returned to the host/patient. These genetically reimplanted cells produce the transfected gene product *in situ*. Alternatively, a xenogenic or allogeneic donor's cells can be genetically modified with the nucleotide sequence *in vitro* and subsequently administered to the patient.

In *in vivo* gene therapy, target cells are not removed from the patient; rather, the gene to be transferred is introduced into the cells of the recipient organism *in situ*, that is within the recipient. Alternatively, if the host gene is defective, the gene is repaired *in situ*. These genetically modified cells produce the transfected gene product *in situ*.

The gene expression vehicle is capable of delivery/transfer of heterologous nucleic acids into a host cell. As indicated previously, the expression vehicle may include elements to control targeting, expression and transcription of the nucleotide sequence in a cell selective or tissue-specific manner, as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene may be replaced by the 5'UTR and/or 3'UTR of the expression vehicle. Therefore as used herein the expression vehicle may, as needed, not include the 5'UTR and/or 3'UTR and only include the specific amino acid coding region.

The expression vehicle can include a promoter for controlling transcription of the heterologous material and can be either a constitutive or inducible promoter to allow selective transcription. Enhancers that may be required to obtain necessary transcription levels can optionally be included. Enhancers are generally any non-translated DNA sequence which works contiguously with the coding sequence (*in cis*) to change the basal transcription level dictated by the promoter. The expression vehicle can also include a selection gene as described herein below.

Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992); in Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley and

Sons, Baltimore, Md. (1989); Chang *et al.*, Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995); Vega *et al.*, Gene Targeting, CRC Press, Ann Arbor, Mich. (1995); Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988); and Gilboa *et al.* (1986) and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Patent Nos. 4,866,042 for vectors involving the central nervous system and also U.S. Patent Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

Introduction of nucleic acids by infection offers several advantages over the other listed methods. Higher efficiency can be obtained due to their infectious nature. Moreover, viruses are very specialized and typically infect and propagate in specific cell types. Thus, their natural specificity can be used to target the vectors to specific cell types, *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

A specific example of a DNA viral vector for introducing and expressing recombinant nucleotide sequences is the adenovirus derived vector Adenop53TK. This vector expresses a herpes virus thymidine kinase (TK) gene for either positive or negative selection and an expression cassette for desired recombinant sequences. This vector can be used to infect cells that have an adenovirus receptor which includes most cancers of epithelial origin as well as others. This vector as well as others that exhibit similar desired functions can be used to treat a mixed population of cells and can include, for example, an *in vitro* or *ex vivo* culture of cells, a tissue or a human subject.

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus. An example of such a negative selection marker is the TK gene described above that confers sensitivity to the antibiotic gancyclovir. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation will not occur. Features that limit expression to particular cell types or tissue types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type or tissue type.

In addition, recombinant viral vectors are useful for *in vivo* expression of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. The vector to be used in the methods of the present invention will depend on desired the cell type or cell types to be targeted and will be known to those skilled in the art. For example, if RSV infection is to be inhibited (*i.e.*, treated or prevented), then a vector specific for such respiratory mucosal epithelial cells would preferably be used.

Retroviral vectors can be constructed to function either as infectious particles or to undergo only a single initial round of infection. In the former case, the genome of the virus is modified so that it maintains all the necessary genes, regulatory sequences and packaging signals to synthesize new viral proteins and RNA. Once these molecules are synthesized, the host cell packages the RNA into new viral particles that are capable of undergoing further rounds of infection. The vector's genome is also engineered to encode and express the desired recombinant nucleotide sequence. In the case of non-infectious viral vectors, the vector genome is usually mutated to destroy the viral packaging signal that is required to encapsulate the RNA into viral particles. Without such a signal, any particles that are formed will not contain a genome and therefore cannot proceed through subsequent rounds of infection. The specific type of vector will depend upon the intended application. The actual vectors are also known and readily available within the art or can be constructed by one skilled in the art using well-known methodology.

The recombinant vector can be administered in several ways. If viral vectors are used, for example, the procedure can take advantage of their target specificity and consequently, do

not have to be administered locally at the diseased site. However, local administration can provide a quicker and more effective treatment, administration can also be performed by, for example, intravenous or subcutaneous injection into the subject. Injection of the viral vectors into a spinal fluid can also be used as a mode of administration, especially in the case of RNA virus infections of the central nervous system. Following injection, the viral vectors will circulate until they recognize host cells with the appropriate target specificity for infection.

An alternate mode of administration can be by direct inoculation locally at the site of the disease or pathological condition or by inoculation into the vascular system supplying the site with nutrients or into the spinal fluid. Local administration is advantageous because there is no dilution effect and, therefore, a smaller dose is required to achieve expression in a majority of the targeted cells. Additionally, local inoculation can alleviate the targeting requirement required with other forms of administration since a vector can be used that infects all cells in the inoculated area. If expression is desired in only a specific subset of cells within the inoculated area, then promoter and regulatory elements that are specific for the desired subset can be used to accomplish this goal. Such non-targeting vectors can be, for example, viral vectors, viral genome, plasmids, phagemids and the like. Transfection vehicles such as liposomes and colloidal polymeric particles can also be used to introduce the non-viral vectors described above into recipient cells within the inoculated area. Such transfection vehicles are known to those skilled within the art.

Direct DNA inoculations can be administered as a method of vaccination. Plasmid DNAs encoding influenza virus hemagglutinin glycoproteins have been tested for the ability to provide protection against lethal influenza challenges. In immunization trials using inoculations of purified DNA in saline, 67-95% of test mice and 25-63% of test chickens were protected against the lethal challenge. Good protection was achieved by intramuscular, intravenous and intradermal injections. In mice, 95% protection was achieved by gene gun delivery of 250-2500 times less DNA than the saline inoculations. Successful DNA vaccination by multiple routes of inoculation and the high efficiency of gene-gun delivery highlight the potential of this promising new approach to immunization. Plasmid DNAs expressing influenza virus hemagglutinin glycoproteins have been tested for their ability to raise protective immunity against lethal influenza challenges of the same subtype. In trials using two inoculations of from 50 to 300 micrograms of purified DNA in saline, 67-95% of test mice and 25-63% of test chickens have been protected against a lethal influenza challenge. Parenteral routes of inoculation that achieved good protection included

intramuscular and intravenous injections. Successful mucosal routes of vaccination included DNA drops administered to the nares or trachea. By far, the most efficient DNA immunizations were achieved by using a gene gun to deliver DNA-coated gold beads to the epidermis. In mice, 95% protection was achieved by two immunizations with beads loaded with as little as 0.4 micrograms of DNA. The breadth of routes supporting successful DNA immunizations, coupled with the very small amounts of DNA required for gene-gun immunizations, highlight the potential of this remarkably simple technique for the development of subunit vaccines. In contrast to the DNA based antigen vaccines, the present invention provides the development of an intranasal gene transfer method using 2-5 AS, or a catalytically active fragment thereof, which can be used as a prophylaxis against multiple respiratory infections. In a preferred embodiment, the preventative and therapeutic method is used against respiratory RNA viral infection, most preferably against RSV.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Claims

What is claimed is:

1. A method of inhibiting an RNA virus infection in a patient by increasing the endogenous 2'-5' oligoadenylate synthetase activity within the patient, wherein the RNA virus is a type that transiently produces double-stranded RNA during intermediate replication.

2. The method according to claim 1, wherein said increasing comprises administering a nucleotide sequence encoding a 2'-5' oligoadenylate synthetase, or a catalytically active fragment thereof, to the patient, wherein the nucleotide sequence is expressed in the patient.

3. The method according to claim 1, wherein said increasing comprises administering a nucleotide sequence encoding at least one catalytically active fragment of 2'-5' oligoadenylate synthetase to the patient, wherein the catalytically active fragment comprises a catalytically active subunit of 2'-5' oligoadenylate synthetase selected from the group consisting of p40, p69, and p100, wherein the nucleotide sequence is expressed in the patient.

4. The method according to any of claims 1-3, wherein the 2'-5' oligoadenylate synthetase is a human enzyme or mammalian homologue.

5. The method according to any of claim 2-4, wherein the nucleotide sequence encodes a polypeptide selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, AND SEQ ID NO: 12, or a catalytically active fragment of any of the foregoing.

6. The method according to any of claims 2-4, wherein the nucleotide sequence comprises at least one sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO:17, or a catalytically active fragment of any of the foregoing.

7. The method according to any of claims 2-6, wherein the nucleotide sequence comprises SEQ ID NO. 1, or a catalytically active fragment thereof.

8. The method according to any of claims 2-6, wherein the nucleotide sequence comprises SEQ ID NO. 3, or a catalytically active fragment thereof.

9. The method according to any of claims 2-6, wherein the nucleotide sequence comprises SEQ ID NO. 5, or a catalytically active fragment thereof.

10. The method according to any of claims 2-6, wherein the nucleotide sequence comprises SEQ ID NO. 7, or a catalytically active fragment thereof.

11. The method according to any of claims 1-10, wherein the RNA virus is a member of the family paramyxoviridae.

12. The method according to any of claims 1-10, wherein the RNA virus is selected from the group consisting of respiratory syncytial virus, rhinovirus, vaccinia virus, reovirus, HIV, EMCV, hepatitis B, hepatitis C, bovine respiratory syncytial virus, measles virus, sendai virus, parainfluenza virus, mumps virus, simian virus, newcastle virus, coronavirus, and West Nile virus.

13. The method according to any of claims 1-10, wherein the RNA virus is coronavirus or West Nile virus.

14. The method according to any of claims 1-13, wherein the RNA virus is one in which exposure to interferon actively inhibits viral replication.

15. The method according to any of claims 1-10, wherein the RNA virus is respiratory syncytial virus.

16. The method according to any of claims 1-15, wherein the patient is human.

17. The method according to any of claims 1-15, wherein the patient is a non-human animal.

18. The method according to any of claims 1-17, wherein the patient is suffering from the RNA virus infection, and wherein the nucleotide sequence alleviates at least one of the symptoms associated with the RNA virus infection.

19. The method according to any of claims 1-17, wherein the patient is not suffering from the RNA virus infection.

20. The method according to any of claims 2-10, wherein the nucleotide sequence is expressed within the patient, thereby eliciting a physiological response from the patient selected from the group consisting of: reduction of respiratory syncytial viral titers within the patient's lungs; reduction of MIP1-α chemokine, decrease in bronchioalveolar lavage lymphocytes and macrophages, reduction in epithelial cell damage, reduction in infiltration of mononuclear cells in the peribronchiolar and perivascular regions, and reduction in thickness of the patient's alveolar septa.

21. The method according to any of claims 2-20, wherein the nucleotide sequence is administered to the patient within a vector, wherein the vector comprises the nucleotide sequence operably linked to a promoter sequence, and wherein the promoter sequence drives expression of the nucleotide sequence.

22. The method according to claim 21, wherein the vector is a viral vector.

23. The method according to claim 22, wherein the viral vector is adenovirus or adeno-associated virus.

24. The method according to claim 21, wherein the vector is a non-viral vector.

25. The method according to claim 21, wherein the vector is a plasmid.

26. The method according to any of claims 2-25, wherein the nucleotide sequence is administered to the patient orally or intranasally.

27. The method according to any of claims 2-26, wherein the nucleotide sequence is administered with a pharmaceutically acceptable carrier.

28. The method according to claim 27, wherein the pharmaceutically acceptable carrier comprises chitosan or a derivative thereof.

29. The method according to claim 1, wherein said increasing comprises administering a 2'-5' oligoadenylate synthetase, or at least one catalytically active fragment thereof, to the patient.

30. The method according to claim 1, wherein said increasing comprises administering at least one catalytically active fragment of 2'-5' oligoadenylate synthetase to the patient, wherein the catalytically active fragment comprises a catalytically active subunit of 2'-5' oligoadenylate synthetase selected from the group consisting of p40, p69, and p100.

31. The method according to claim 29, wherein the 2'-5' oligoadenylate synthetase is a human enzyme or mammalian homologue.

32. The method according to claim 29, wherein a catalytically active fragment of 2'-5' oligoadenylate synthetase is administered to the patient, wherein the catalytically active fragment comprises at least one amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12, or a catalytically active fragment of any of the foregoing.

33. The method according to claim 32, wherein the amino acid sequence administered to the patient comprises SEQ ID NO: 2, or a catalytically active fragment thereof.

34. The method according to claim 32, wherein the amino acid sequence administered to the patient comprises SEQ ID NO: 4, or a catalytically active fragment thereof.

35. The method according to claim 32, wherein the amino acid sequence administered to the patient comprises SEQ ID NO: 6, or a catalytically active fragment thereof.

36. The method according to claim 32, wherein the amino acid sequence administered to the patient comprises SEQ ID NO: 8, or a catalytically active fragment thereof

37. The method according to claim 29, wherein the 2'-5' oligoadenylate synthetase, or at least one catalytically active fragment thereof, is administered to the patient with a pharmaceutically acceptable carrier.

38. The method according to claim 29, wherein the 2'-5' oligoadenylate synthetase, or at least one catalytically active fragment thereof, is administered to the patient within a composition that protects the 2'-5' oligoadenylate synthetase, or at least one catalytically active fragment thereof, from premature proteolytic degradation within the patient.

39. A pharmaceutical composition comprising a nucleotide sequence encoding a 2'-5' oligoadenylate synthetase, or at least one catalytically active fragment thereof, and a pharmaceutically acceptable carrier.

40. The composition of claim 39, wherein said composition further comprises an anti-viral agent.

41. The pharmaceutical composition of claim 39, wherein said nucleotide sequence encodes a polypeptide selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, AND SEQ ID NO: 12, or a catalytically active fragment of any of the foregoing.

42. The pharmaceutical composition of claim 39, wherein the nucleotide sequence comprises at least one sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16, or a catalytically active fragment of any of the foregoing.

43. A pharmaceutical composition comprising a 2'-5' oligoadenylate synthetase, or at least one catalytically active fragment thereof, and a pharmaceutically acceptable carrier.

44. The pharmaceutical composition of claim 43, wherein the composition comprises a polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12, or a catalytically active fragment of the foregoing.

45. A vector comprising a nucleotide sequence encoding a 2'-5' oligoadenylate synthetase, or at least one catalytically active fragment thereof; and a promoter sequence operably linked to said nucleotide sequence.

46. The vector of claim 46, wherein said nucleotide sequence encodes a polypeptide selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, AND SEQ ID NO: 12, or a catalytically active fragment of any of the foregoing.

47. The vector of claim 45, wherein said nucleotide sequence comprises at least one sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16, or a catalytically active fragment of any of the foregoing.

48. The vector of claim 45, wherein said vector is a viral vector.

47. The vector of claim 45, wherein said viral vector is an adenoviral vector or adeno-associated viral vector.

48. A host cell that has been genetically modified with a nucleotide sequence encoding a 2'-5' oligoadenylate synthetase, or at least one catalytically active fragment thereof, wherein said nucleotide sequence is expressed in said cell.

49. The host cell of claim 48, wherein said nucleotide sequence encodes a polypeptide selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, AND SEQ ID NO: 12, or a catalytically active fragment of any of the foregoing.

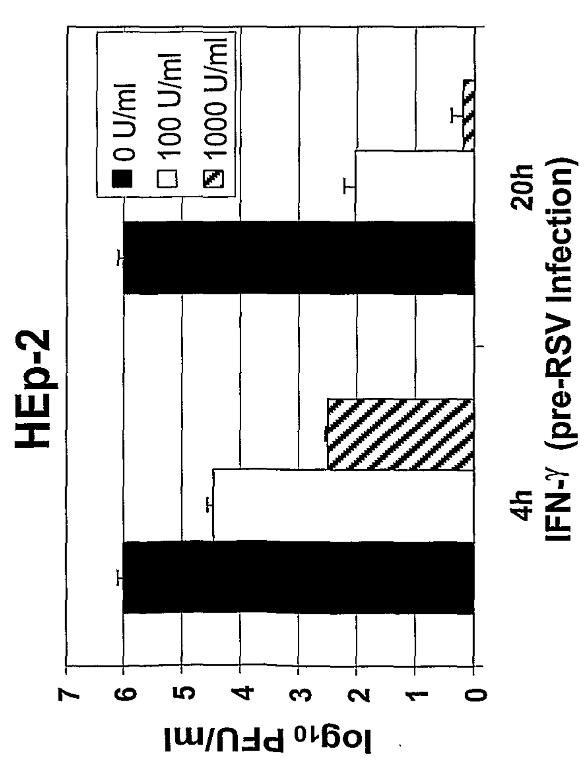
50. The host cell of claim 48, wherein said nucleotide sequence comprises at least one sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16, or a catalytically active fragment of any of the foregoing.

51. The host cell of claim 48, wherein said host cell is a prokaryotic cell.

52. The host cell of claim 48, wherein said host cell is a eukaryotic cell.

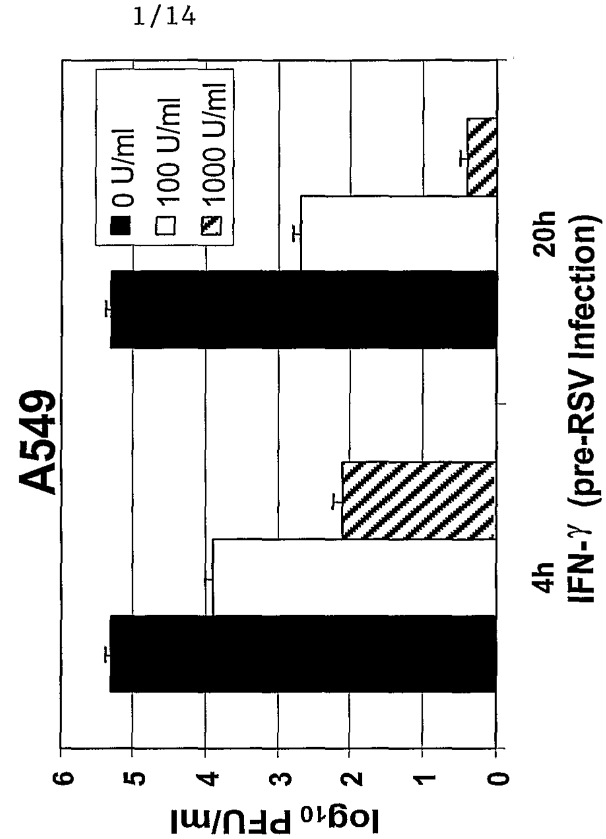
53. The host cell of claim 48, wherein said host cell is a mammalian cell.

54. The host cell of claim 48, wherein said host cell is a human cell.



HEp-2
log10 PFU/ml
7
6
5
4
3
2
1
0
0 U/ml
100 U/ml
1000 U/ml
4h
20h
IFN-γ (pre-RSV Infection)

FIG. 1A



A549
log10 PFU/ml
6
5
4
3
2
1
0
0 U/ml
100 U/ml
1000 U/ml
4h
20h
IFN-γ (pre-RSV Infection)

FIG. 1B

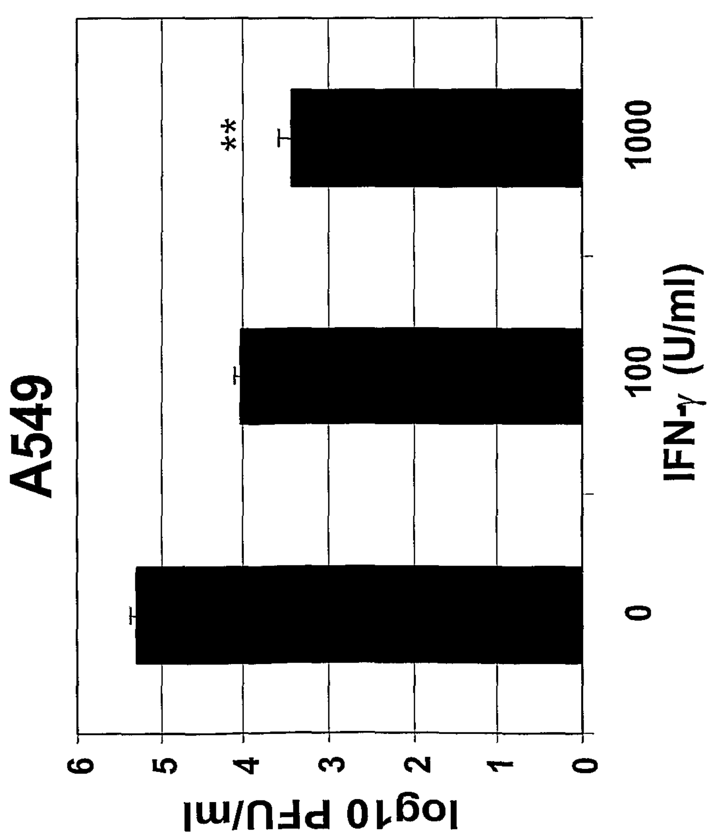

A549
log10 PFU/ml
0
1
2
3
4
5
6
**
0
100
1000
IFN-γ (U/ml)

FIG. 1C

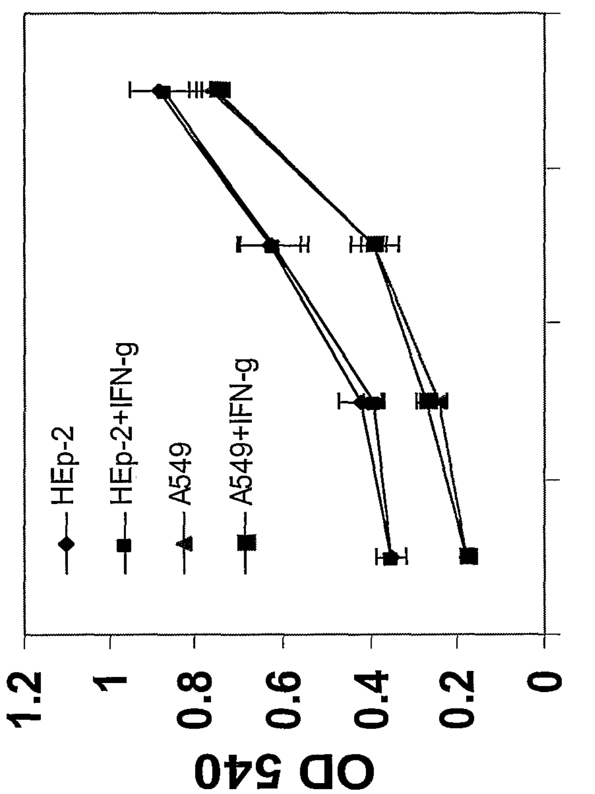

OD 540
0
0.2
0.4
0.6
0.8
1
1.2
HEp-2
HEp-2+IFN-g
A549
A549+IFN-g
0h
24h
48h
72h

FIG. 1D

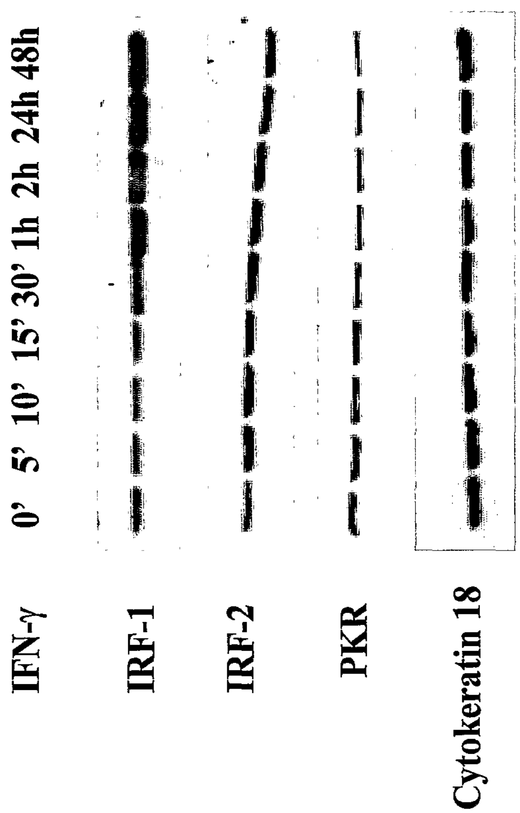

IFN-γ
0' 5' 10' 15' 30' 1h 2h 24h 48h
IRF-1
IRF-2
PKR
Cytokeratin 18

FIG. 2A

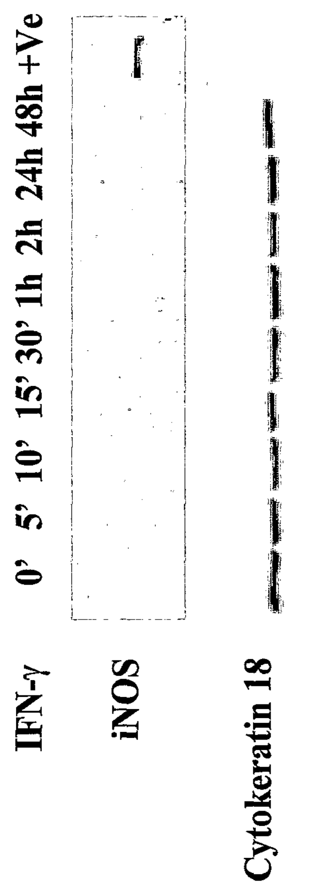

IFN-γ
0' 5' 10' 15' 30' 1h 2h 24h 48h +Ve
iNOS
Cytokeratin 18

FIG. 2B

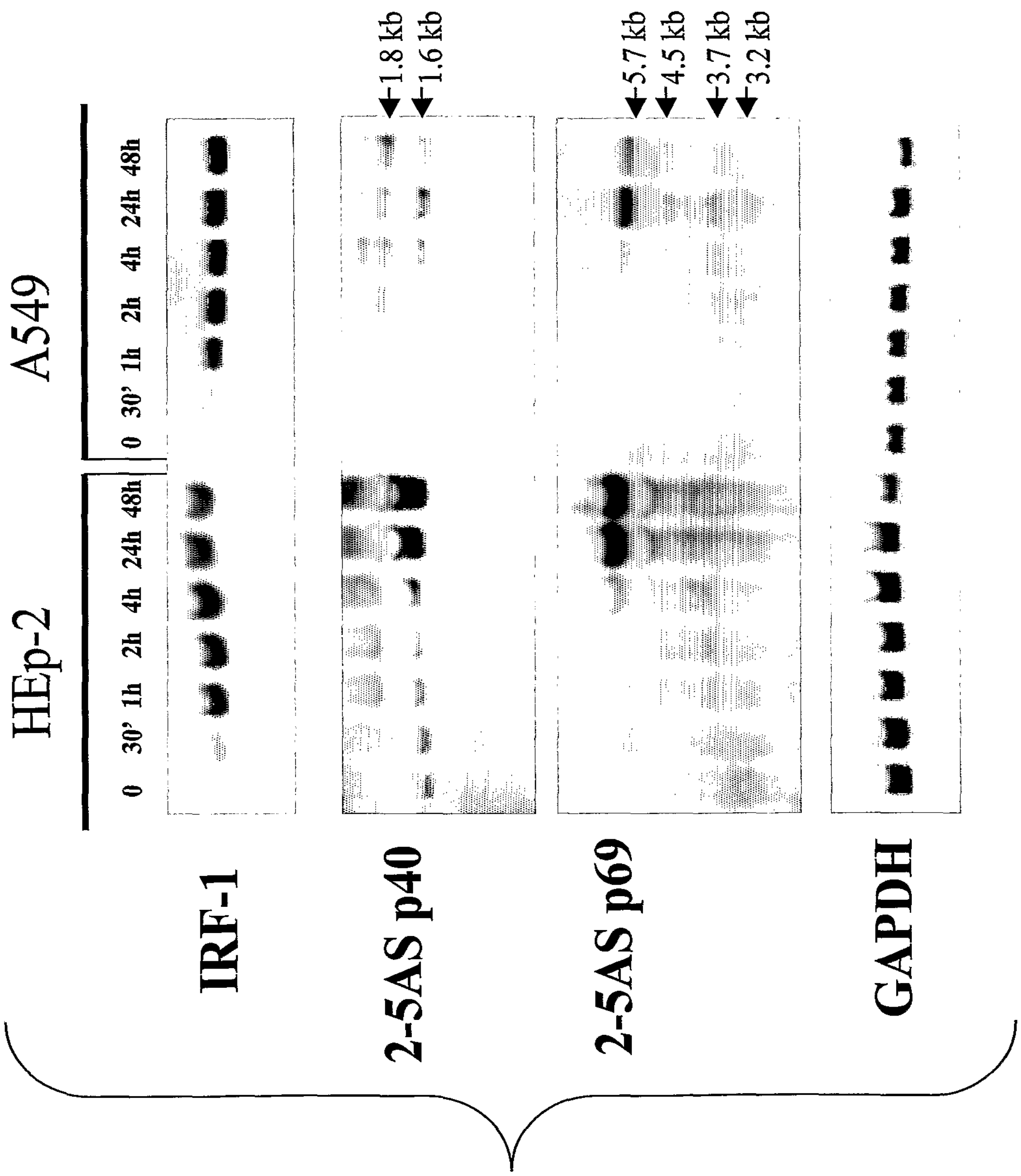

HEp-2
A549
0 30' 1h 2h 4h 24h 48h 0 30' 1h 2h 4h 24h 48h
IRF-1
2-5AS p40
2-5AS p69
GAPDH
1.8 kb
1.6 kb
5.7 kb
4.5 kb
3.7 kb
3.2 kb

FIG. 3

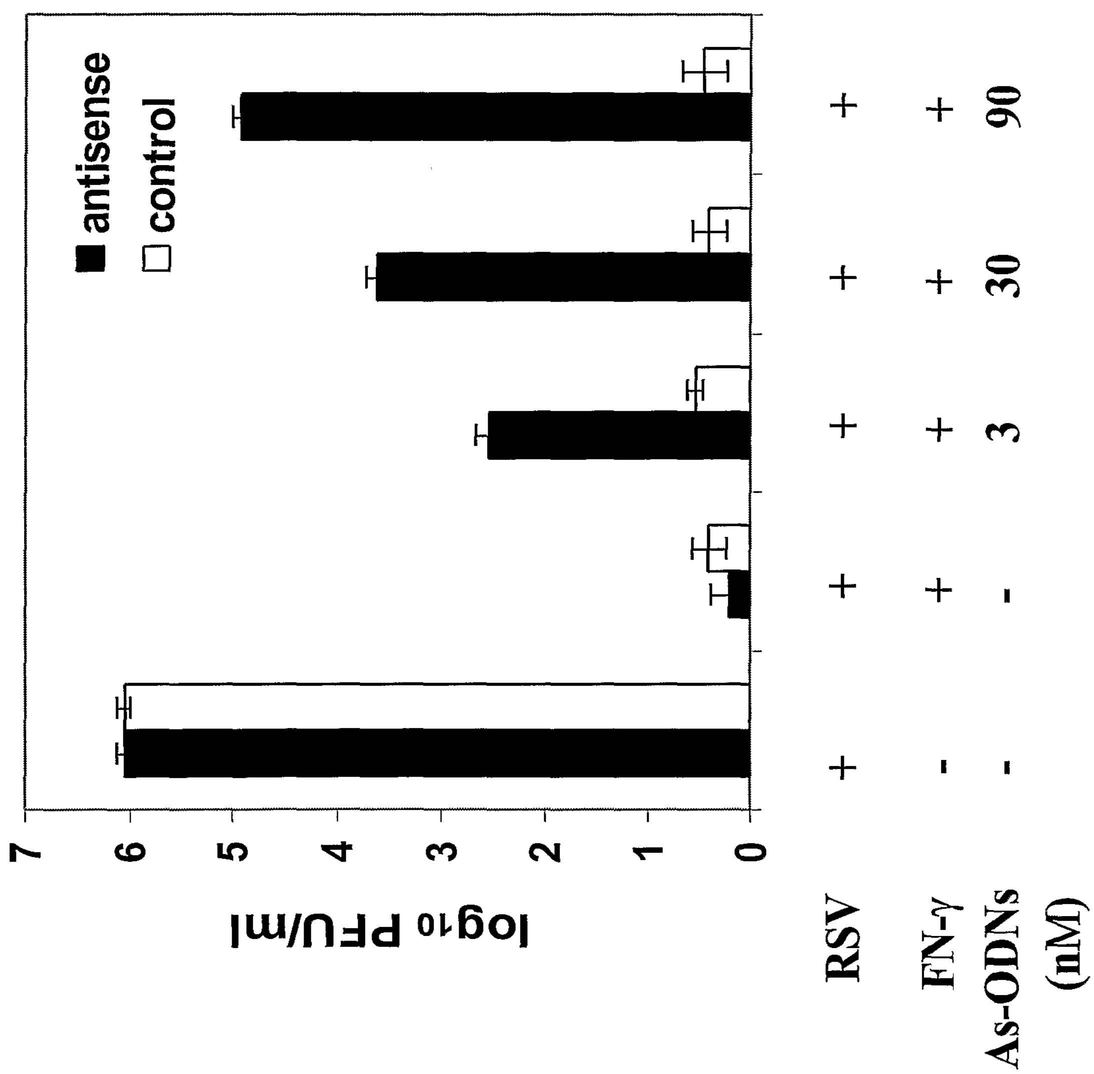

log10 PFU/ml
0
1
2
3
4
5
6
7
antisense
control
RSV + + + + +
FN-γ - + + + +
As-ODNs (nM) - - 3 30 90

FIG. 4

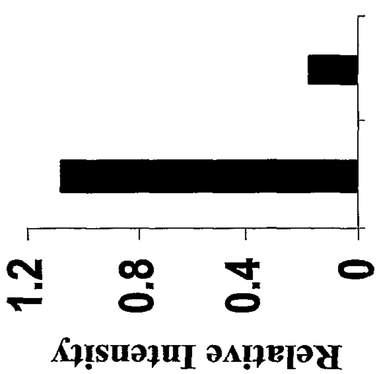


FIG. 5B

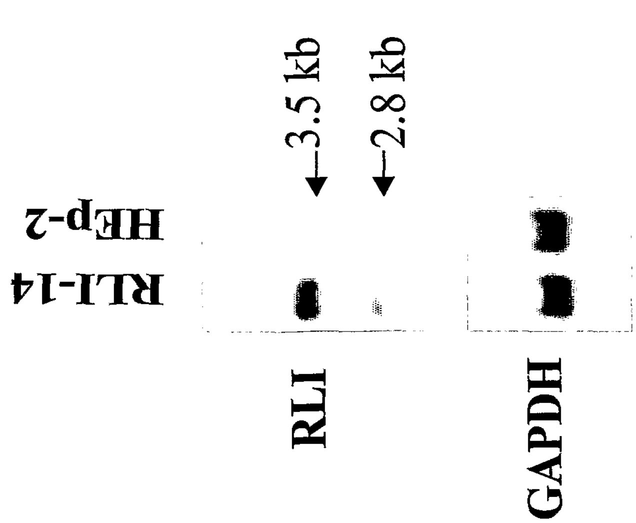


FIG. 5A

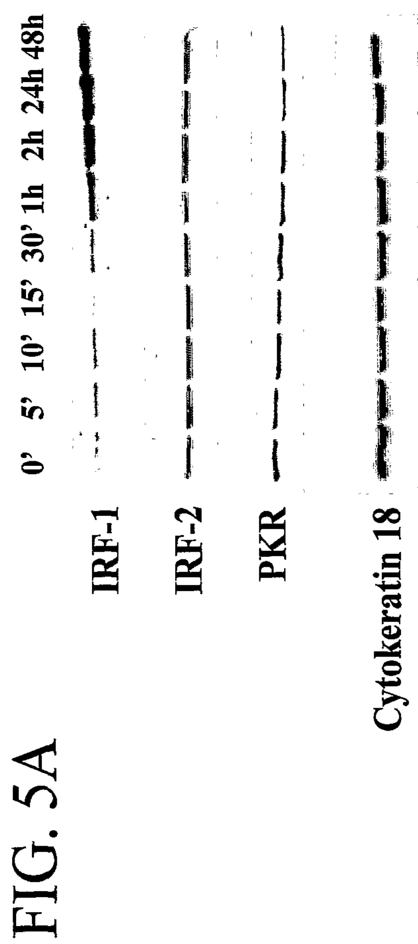


FIG. 5C

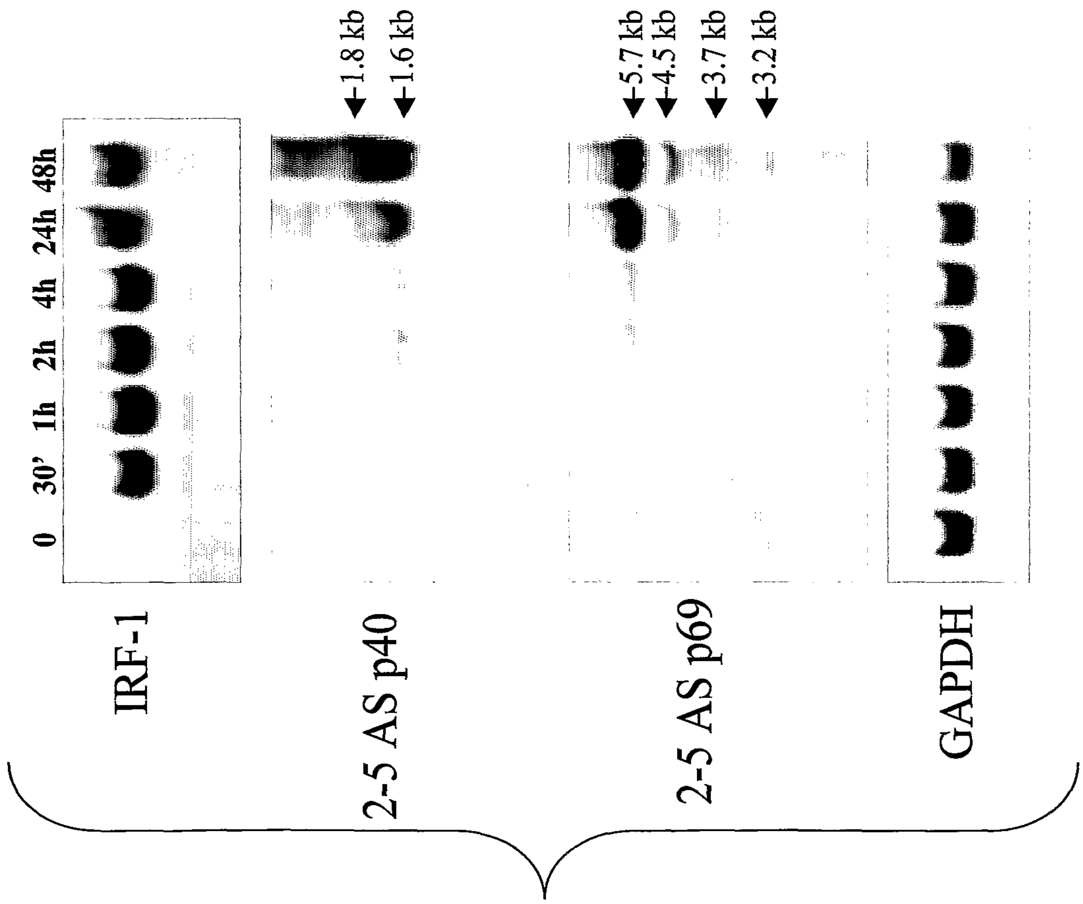

IRF-1
2-5 AS p40
2-5 AS p69
GAPDH
0
30'
1h
2h
4h
24h
48h
1.8 kb
1.6 kb
5.7 kb
4.5 kb
3.7 kb
3.2 kb

FIG. 5D

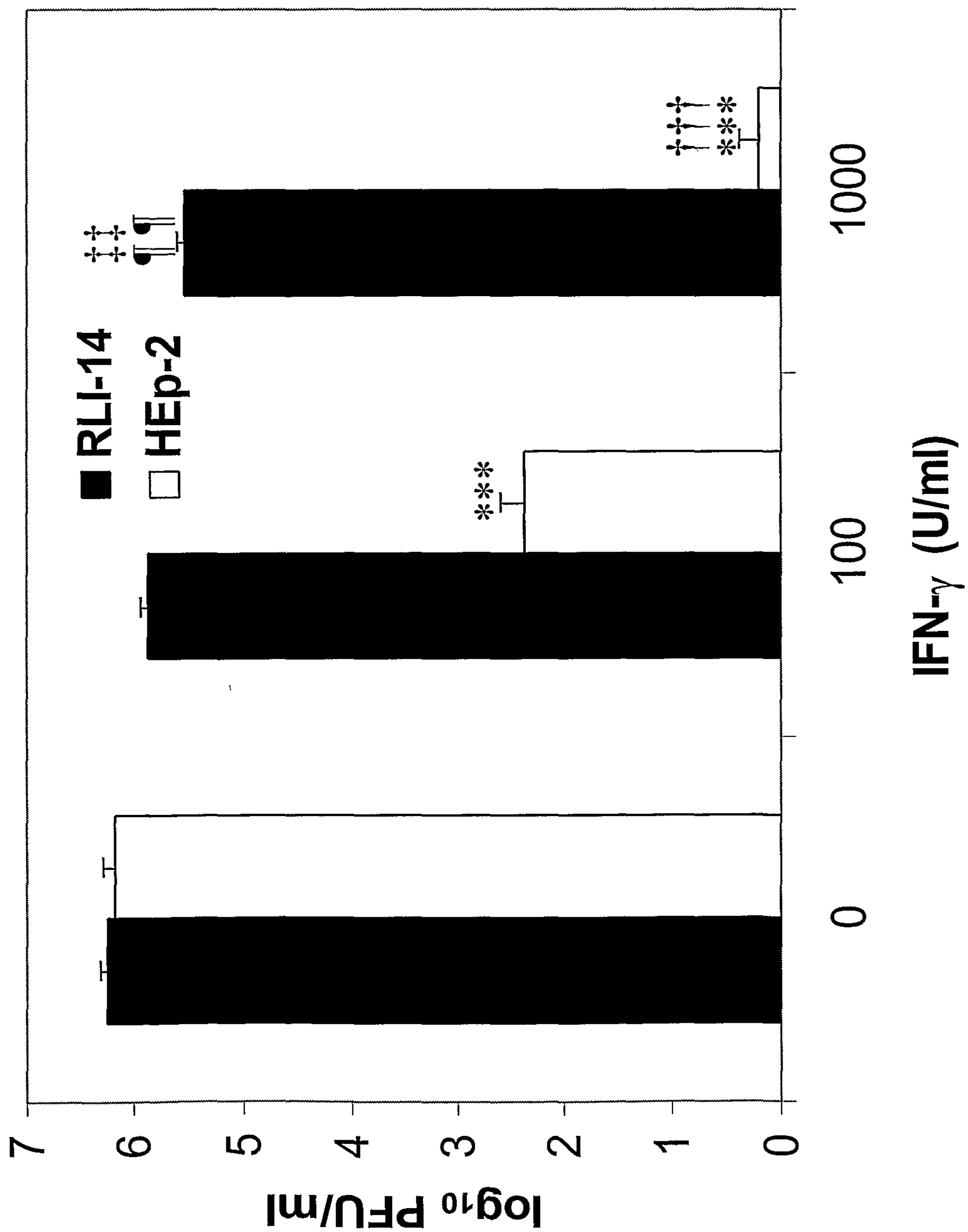
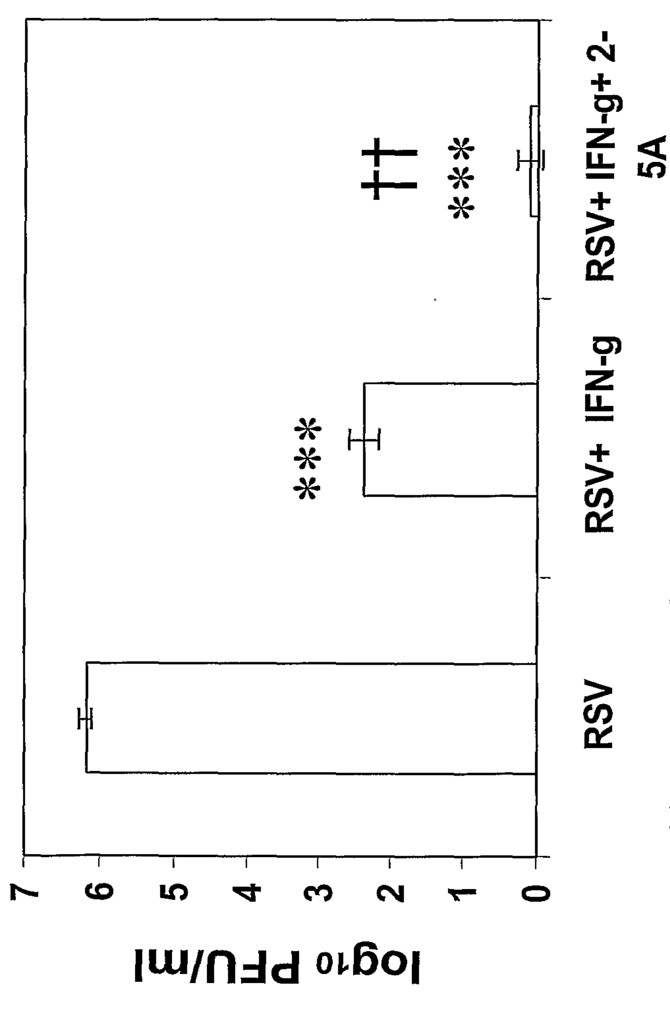


FIG. 6



***: $p < 0.005$; ††: $p < 0.05$.

FIG. 7

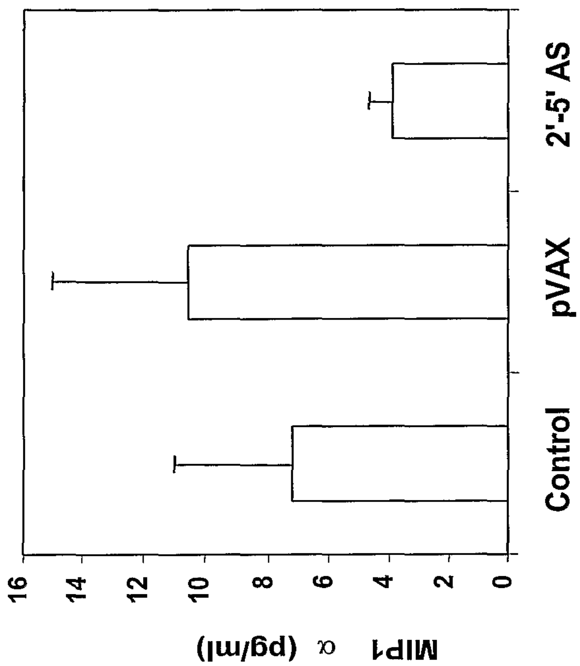


FIG. 8B

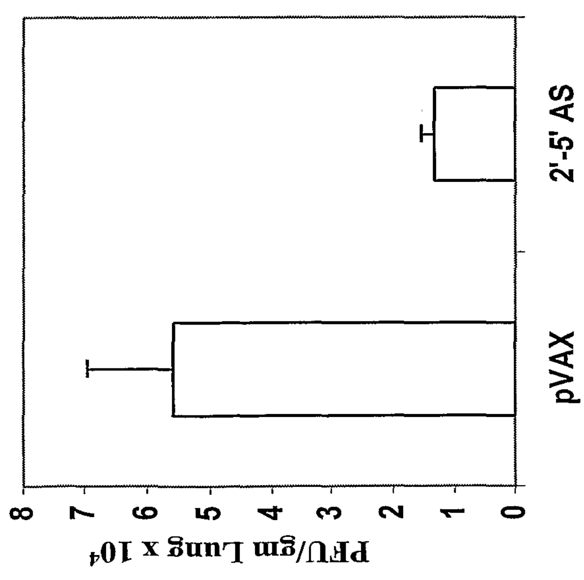


FIG. 8A

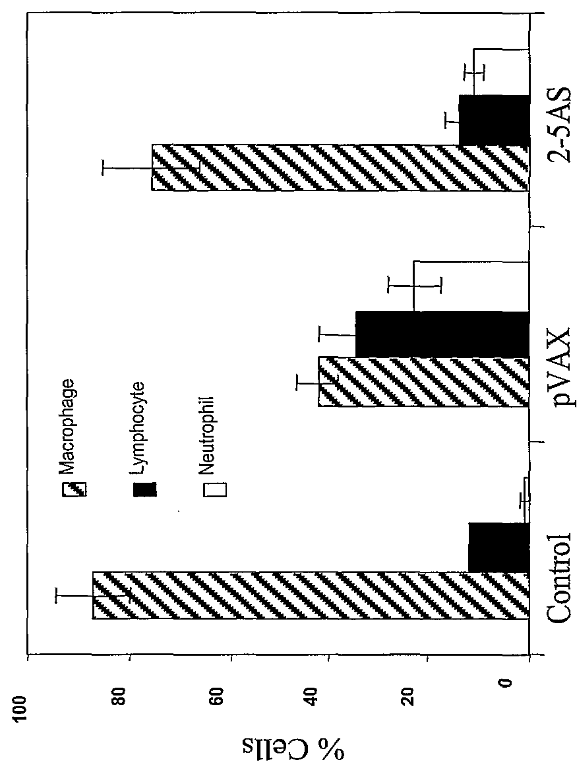


FIG. 8C

Control

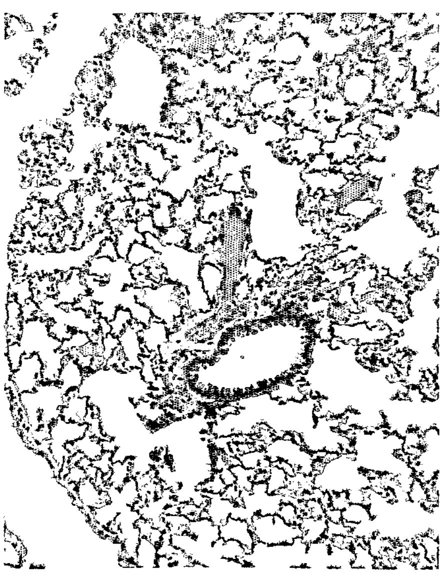


FIG. 9A

pVAX+RSV

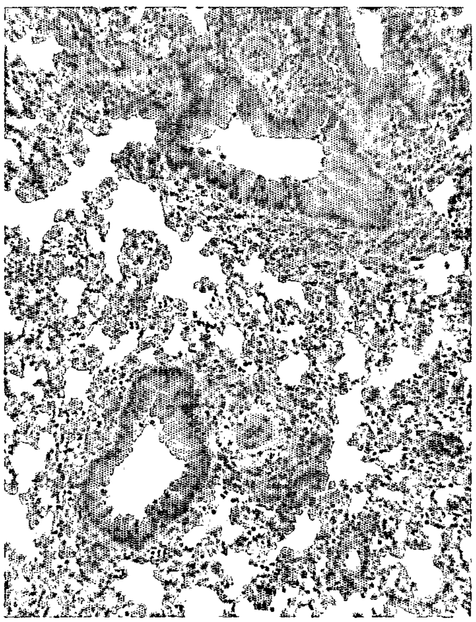


FIG. 9B

p2'-5' AS+RSV

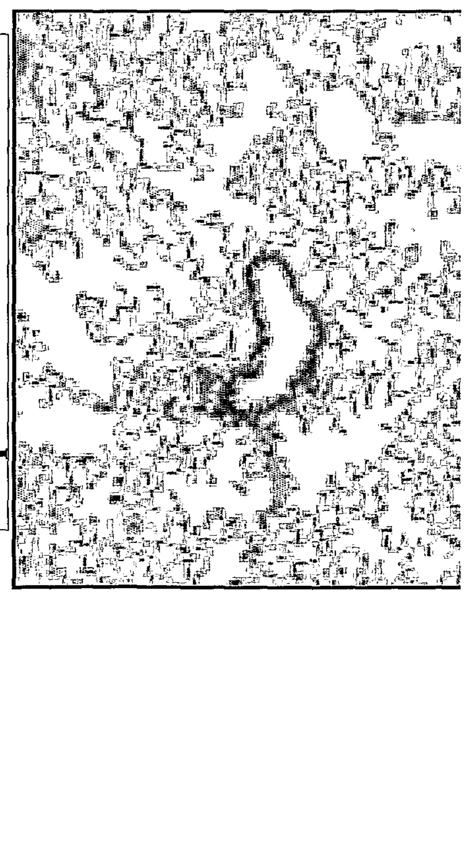
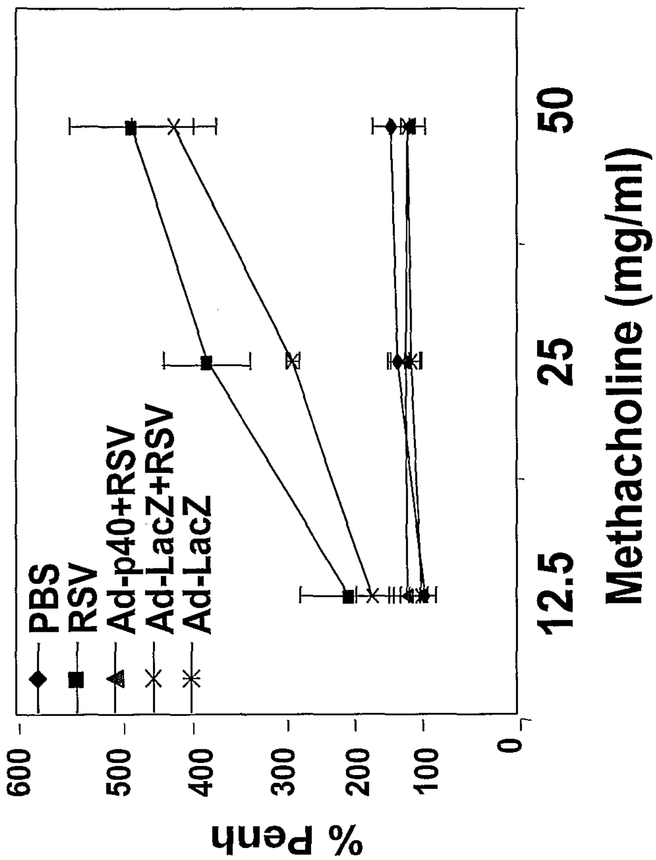
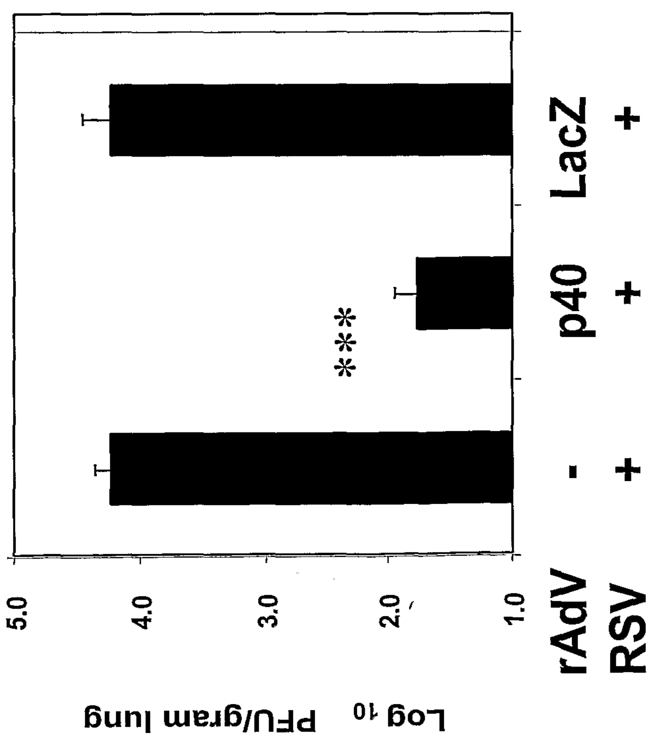


FIG. 9C



% Penh
600
500
400
300
200
100
0
PBS
RSV
Ad-p40+RSV
Ad-LacZ+RSV
Ad-LacZ
12.5
25
50
Methacholine (mg/ml)

FIG. 11



Log 10 PFU/gram lung
5.0
4.0
3.0
2.0
1.0
***
rAdV
RSV
-
+
p40
+
LacZ
+

FIG. 10

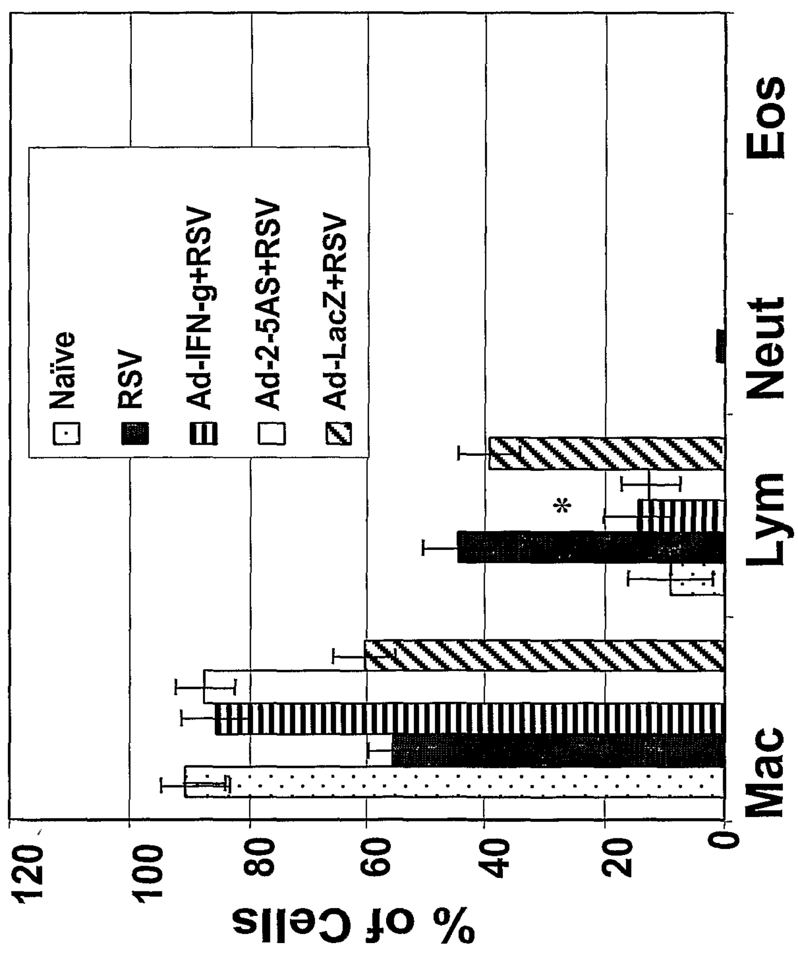

Naïve
RSV
Ad-IFN-g+RSV
Ad-2-5AS+RSV
Ad-LacZ+RSV
% of Cells
120
100
80
60
40
20
0
*
Mac
Lym
Neut
Eos

FIG. 12

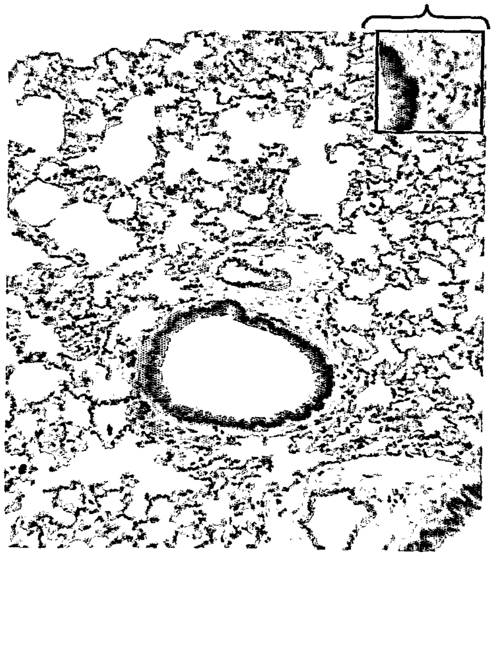


FIG. 13A

FIG. 13B

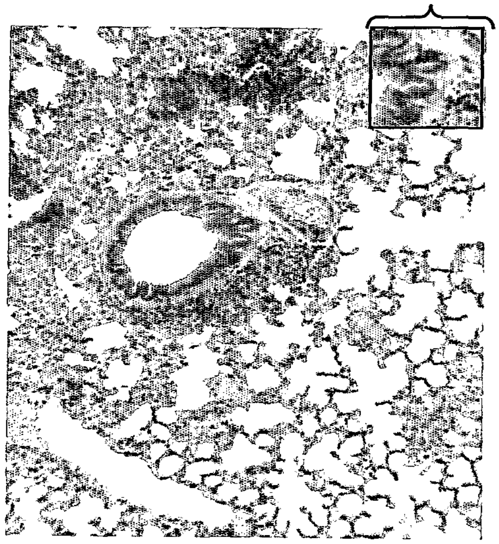


FIG. 13C

FIG. 13D

FIG. 13E

FIG. 13F

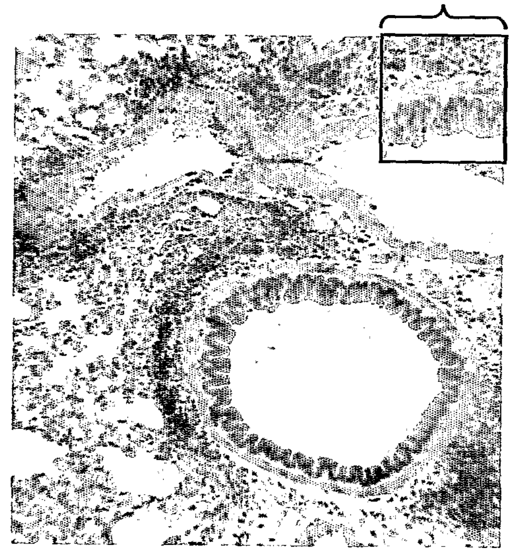


FIG. 13G

FIG. 13H

SEQUENCE LISTING

<110> University of South Florida

<120> Materials and Methods for Prevention and Treatment of RNA Viral Diseases

<130> USF-168XC1

<150> US 60/319,216
<151> 2002-04-30

<150> US 60/319,313
<151> 2002-06-12

<160> 20

<170> PatentIn version 3.1

<210> 1
<211> 1203
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1203)
<223>

<300>
<301> Aissouni, Y., et al.
<302> The cleavage/polyadenylation activity triggered by a U-rich motif sequence
<303> J. Biol. Chem.
<304> 277
<305> 39
<306> 35808-35814
<307> 2002
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Behera, A.K., et al.
<302> 2'-5' Oligoadenylate synthesis plays a critical role in interferon-gamma inhibition
<303> J. Biol. Chem.
<304> 277
<305> 28
<306> 25601-25608
<307> 2002
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Sarker, S.N., et al.
<302> Identification of the substrate-binding sites of 2'-5'-oligoadenylate synthetase
<303> J. Biol. Chem.
<304> 277
<305> 27

<306> 24321-24330
<307> 2002
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Hovnanian, A., et al.
<302> The human 2',5'-oligoadenylate synthetase locus is composed of three distinct genes
<303> Genomics
<304> 52
<305> 3
<306> 267-277
<307> 1998
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Renault, B., et al.
<302> A sequence-ready physical map of a region of 12q24.1
<303> Genomics
<304> 45
<305> 2
<306> 271-278
<307> 1997
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Nechiporuk, T., et al.
<302> A high-resolution PAC and BAC map of the SCA2 region
<303> Genomics
<304> 44
<305> 3
<306> 321-329
<307> 1997
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Wathelet, M.G., et al.
<302> Cloning and chromosomal location of human genes inducible by type I interferon
<303> Somat. Cell Mol. Genet.
<304> 14
<305> 5
<306> 415-426
<307> 1988
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Rutherford, M.N., et al.
<302> Interferon-induced binding of nuclear factors to promoter elements of the 2-5A synthetase gene
<303> EMBO J.
<304> 7
<305> 3
<306> 751-759
<307> 1988
<308> NCBI/NM_016816

<309> 2003-04-06

<300>
<301> Wathelet, M.G., et al.
<302> New inducers revealed by the promoter sequence analysis of two interferon-activated human genes
<303> Eur. J. Biochem.
<304> 169
<305> 2
<306> 313-321
<307> 1987
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Benech, P., et al.
<302> Interferon-responsive regulatory elements in the promoter of the human 2',5'-oligo(A) synthetase gene
<303> Mol. Cell. Biol.
<304> 7
<305> 12
<306> 4498-4504
<307> 1987
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Hovanessian, A.G., et al.
<302> Identification of 69-kd and 100-kd forms of 2-5A synthetase
<303> EMBO J.
<304> 6
<305> 5
<306> 1273-1280
<307> 1987
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Williams, B.R., et al.
<302> Interferon-regulated human 2-5A synthetase gene maps to chromosome
<303> Somat. Cell Mol. Genet.
<304> 12
<305> 4
<306> 403-408
<307> 1986
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Shiojiri, S., et al.
<302> Structure and expression of a cloned cDNA for human (2'-5') oligoadenylate synthetase
<303> J. Biochem.
<304> 99
<305> 5
<306> 1455-1464
<307> 1986
<308> NCBI/NM_016816
<309> 2003-04-06

<300>

<301> Wathelet, M., et al.
<302> Full-length sequence and expression of the 42 kDa 2-5A synthetase
<303> FEBS Lett.
<304> 196
<305> 1
<306> 113-120
<307> 1986
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Benech, P., et al.
<302> Structure of two forms of the interferon-induced (2'-5') oligo A synthetase of human cells
<303> EMBO J.
<304> 4
<305> 9
<306> 2249-2256
<307> 1985
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Saunders, M.E., et al.
<302> Human 2-5A synthetase: characterization of a novel cDNA and corresponding gene structure
<303> EMBO J.
<304> 4
<305> 7
<306> 1761-1768
<307> 1985
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Merlin, G., et al.
<302> Molecular cloning and sequence of partial cDNA for interferon-induced (2'-5')oligo(A_ synthetase mRNA from human cells
<303> Proc. Natl. Acad. Sci. U.S.A.
<304> 80
<305> 16
<306> 4904-4908
<307> 1983
<308> NCBI/NM_016816
<309> 2003-04-06

<400> 1
atg atg gat ctc aga aat acc cca gcc aaa tct ctg gac aag ttc att 48
Met Met Asp Leu Arg Asn Thr Pro Ala Lys Ser Leu Asp Lys Phe Ile
1 5 10 15

gaa gac tat ctc ttg cca gac acg tgt ttc cgc atg caa atc gac cat 96
Glu Asp Tyr Leu Leu Pro Asp Thr Cys Phe Arg Met Gln Ile Asp His
20 25 30

gcc att gac atc atc tgt ggg ttc ctg aag gaa agg tgc ttc cga ggt 144
Ala Ile Asp Ile Ile Cys Gly Phe Leu Lys Glu Arg Cys Phe Arg Gly
35 40 45

agc tcc tac cct gtg tgt gtg tcc aag gtg gta aag ggt ggc tcc tca 192
Ser Ser Tyr Pro Val Cys Val Ser Lys Val Val Lys Gly Gly Ser Ser

```
    50                  55                  60

ggc aag ggc acc acc ctc aga ggc cga tct gac gct gac ctg gtt gtc       240
Gly Lys Gly Thr Thr Leu Arg Gly Arg Ser Asp Ala Asp Leu Val Val
65                  70                  75                  80

ttc ctc agt cct ctc acc act ttt cag gat cag tta aat cgc cgg gga       288
Phe Leu Ser Pro Leu Thr Thr Phe Gln Asp Gln Leu Asn Arg Arg Gly
                85                  90                  95

gag ttc atc cag gaa att agg aga cag ctg gaa gcc tgt caa aga gag       336
Glu Phe Ile Gln Glu Ile Arg Arg Gln Leu Glu Ala Cys Gln Arg Glu
            100                 105                 110

aga gca ctt tcc gtg aag ttt gag gtc cag gct cca cgc tgg ggc aac       384
Arg Ala Leu Ser Val Lys Phe Glu Val Gln Ala Pro Arg Trp Gly Asn
        115                 120                 125

ccc cgt gcg ctc agc ttc gta ctg agt tcg ctc cag ctc ggg gag ggg       432
Pro Arg Ala Leu Ser Phe Val Leu Ser Ser Leu Gln Leu Gly Glu Gly
    130                 135                 140

gtg gag ttc gat gtg ctg cct gcc ttt gat gcc ctg ggt cag ttg act       480
Val Glu Phe Asp Val Leu Pro Ala Phe Asp Ala Leu Gly Gln Leu Thr
145                 150                 155                 160

ggc agc tat aaa cct aac ccc caa atc tat gtc aag ctc atc gag gag       528
Gly Ser Tyr Lys Pro Asn Pro Gln Ile Tyr Val Lys Leu Ile Glu Glu
                165                 170                 175

tgc acc gac ctg cag aaa gag ggc gag ttc tcc acc tgc ttc aca gaa       576
Cys Thr Asp Leu Gln Lys Glu Gly Glu Phe Ser Thr Cys Phe Thr Glu
            180                 185                 190

cta cag aga gac ttc ctg aag cag cgc ccc acc aag ctc aag agc ctc       624
Leu Gln Arg Asp Phe Leu Lys Gln Arg Pro Thr Lys Leu Lys Ser Leu
        195                 200                 205

atc cgc cta gtc aag cac tgg tac caa aat tgt aag aag aag ctt ggg       672
Ile Arg Leu Val Lys His Trp Tyr Gln Asn Cys Lys Lys Lys Leu Gly
    210                 215                 220

aag ctg cca cct cag tat gcc ctg gag ctc ctg acg gtc tat gct tgg       720
Lys Leu Pro Pro Gln Tyr Ala Leu Glu Leu Leu Thr Val Tyr Ala Trp
225                 230                 235                 240

gag cga ggg agc atg aaa aca cat ttc aac aca gcc caa gga ttt cgg       768
Glu Arg Gly Ser Met Lys Thr His Phe Asn Thr Ala Gln Gly Phe Arg
                245                 250                 255

acg gtc ttg gaa tta gtc ata aac tac cag caa ctc tgc atc tac tgg       816
Thr Val Leu Glu Leu Val Ile Asn Tyr Gln Gln Leu Cys Ile Tyr Trp
            260                 265                 270

aca aag tat tat gac ttt aaa aac ccc att att gaa aag tac ctg aga       864
Thr Lys Tyr Tyr Asp Phe Lys Asn Pro Ile Ile Glu Lys Tyr Leu Arg
        275                 280                 285

agg cag ctc acg aaa ccc agg cct gtg atc ctg gac ccg gcg gac cct       912
Arg Gln Leu Thr Lys Pro Arg Pro Val Ile Leu Asp Pro Ala Asp Pro
    290                 295                 300
```

```
aca gga aac ttg ggt ggt gga gac cca aag ggt tgg agg cag ctg gca        960
Thr Gly Asn Leu Gly Gly Gly Asp Pro Lys Gly Trp Arg Gln Leu Ala
305                 310                 315                 320

caa gag gct gag gcc tgg ctg aat tac cca tgc ttt aag aat tgg gat       1008
Gln Glu Ala Glu Ala Trp Leu Asn Tyr Pro Cys Phe Lys Asn Trp Asp
                325                 330                 335

ggg tcc cca gtg agc tcc tgg att ctg ctg gct gaa agc aac agt aca       1056
Gly Ser Pro Val Ser Ser Trp Ile Leu Leu Ala Glu Ser Asn Ser Thr
            340                 345                 350

gac gat gag acc gac gat ccc agg acg tat cag aaa tat ggt tac att       1104
Asp Asp Glu Thr Asp Asp Pro Arg Thr Tyr Gln Lys Tyr Gly Tyr Ile
        355                 360                 365

gga aca cat gag tac cct cat ttc tct cat aga ccc agc acg ctc cag       1152
Gly Thr His Glu Tyr Pro His Phe Ser His Arg Pro Ser Thr Leu Gln
    370                 375                 380

gca gca tcc acc cca cag gca gaa gag gac tgg acc tgc acc atc ctc       1200
Ala Ala Ser Thr Pro Gln Ala Glu Glu Asp Trp Thr Cys Thr Ile Leu
385                 390                 395                 400

tga                                                                   1203


<210>  2
<211>  400
<212>  PRT
<213>  Homo sapiens

<400>  2

Met Met Asp Leu Arg Asn Thr Pro Ala Lys Ser Leu Asp Lys Phe Ile
1               5                   10                  15


Glu Asp Tyr Leu Leu Pro Asp Thr Cys Phe Arg Met Gln Ile Asp His
            20                  25                  30


Ala Ile Asp Ile Ile Cys Gly Phe Leu Lys Glu Arg Cys Phe Arg Gly
        35                  40                  45


Ser Ser Tyr Pro Val Cys Val Ser Lys Val Val Lys Gly Gly Ser Ser
    50                  55                  60


Gly Lys Gly Thr Thr Leu Arg Gly Arg Ser Asp Ala Asp Leu Val Val
65                  70                  75                  80


Phe Leu Ser Pro Leu Thr Thr Phe Gln Asp Gln Leu Asn Arg Arg Gly
                85                  90                  95


Glu Phe Ile Gln Glu Ile Arg Arg Gln Leu Glu Ala Cys Gln Arg Glu
            100                 105                 110
```

```
Arg Ala Leu Ser Val Lys Phe Glu Val Gln Ala Pro Arg Trp Gly Asn
        115                 120                 125

Pro Arg Ala Leu Ser Phe Val Leu Ser Ser Leu Gln Leu Gly Glu Gly
    130                 135                 140

Val Glu Phe Asp Val Leu Pro Ala Phe Asp Ala Leu Gly Gln Leu Thr
145                 150                 155                 160

Gly Ser Tyr Lys Pro Asn Pro Gln Ile Tyr Val Lys Leu Ile Glu Glu
                165                 170                 175

Cys Thr Asp Leu Gln Lys Glu Gly Glu Phe Ser Thr Cys Phe Thr Glu
            180                 185                 190

Leu Gln Arg Asp Phe Leu Lys Gln Arg Pro Thr Lys Leu Lys Ser Leu
        195                 200                 205

Ile Arg Leu Val Lys His Trp Tyr Gln Asn Cys Lys Lys Lys Leu Gly
    210                 215                 220

Lys Leu Pro Pro Gln Tyr Ala Leu Glu Leu Leu Thr Val Tyr Ala Trp
225                 230                 235                 240

Glu Arg Gly Ser Met Lys Thr His Phe Asn Thr Ala Gln Gly Phe Arg
                245                 250                 255

Thr Val Leu Glu Leu Val Ile Asn Tyr Gln Gln Leu Cys Ile Tyr Trp
            260                 265                 270

Thr Lys Tyr Tyr Asp Phe Lys Asn Pro Ile Ile Glu Lys Tyr Leu Arg
        275                 280                 285

Arg Gln Leu Thr Lys Pro Arg Pro Val Ile Leu Asp Pro Ala Asp Pro
    290                 295                 300

Thr Gly Asn Leu Gly Gly Gly Asp Pro Lys Gly Trp Arg Gln Leu Ala
305                 310                 315                 320

Gln Glu Ala Glu Ala Trp Leu Asn Tyr Pro Cys Phe Lys Asn Trp Asp
                325                 330                 335

Gly Ser Pro Val Ser Ser Trp Ile Leu Leu Ala Glu Ser Asn Ser Thr
            340                 345                 350
```

```
Asp Asp Glu Thr Asp Asp Pro Arg Thr Tyr Gln Lys Tyr Gly Tyr Ile
        355                 360                 365

Gly Thr His Glu Tyr Pro His Phe Ser His Arg Pro Ser Thr Leu Gln
    370                 375                 380

Ala Ala Ser Thr Pro Gln Ala Glu Glu Asp Trp Thr Cys Thr Ile Leu
385                 390                 395                 400

<210>  3
<211>  1207
<212>  DNA
<213>  Homo sapiens

<220>
<221>  CDS
<222>  (1)..(1206)
<223>

<300>
<301>  Benech et al.
<302>  Structure of two forms of the interferon-induced (2'-5') oligo A
synthetase human cells based on cDNAs and gene sequences
<303>  EMBO J.
<304>  4
<305>  9
<306>  2249-2256
<307>  1985
<308>  NM_016816
<309>  2003-04-06

<400>  3
atg atg gat ctc aga aat acc cca gcc aaa tct ctg gac aag ttc att        48
Met Met Asp Leu Arg Asn Thr Pro Ala Lys Ser Leu Asp Lys Phe Ile
1               5                   10                  15

gaa gac tat ctc ttg cca gac acg tgt ttc cgc atg caa atc gac cat        96
Glu Asp Tyr Leu Leu Pro Asp Thr Cys Phe Arg Met Gln Ile Asp His
            20                  25                  30

gcc att gac atc atc tgt ggg ttc ctg aag gaa agg tgc ttc cga ggt       144
Ala Ile Asp Ile Ile Cys Gly Phe Leu Lys Glu Arg Cys Phe Arg Gly
        35                  40                  45

agc tcc tac cct gtg tgt gtg tcc aag gtg gta aag ggt ggc tcc tca       192
Ser Ser Tyr Pro Val Cys Val Ser Lys Val Val Lys Gly Gly Ser Ser
    50                  55                  60

ggc aag ggc acc acc ctc aga ggc cga tct gac gct gac ctg gtt gtc       240
Gly Lys Gly Thr Thr Leu Arg Gly Arg Ser Asp Ala Asp Leu Val Val
65                  70                  75                  80

ttc ctc agt cct ctc acc act ttt cag gat cag tta aat cgc cgg gga       288
Phe Leu Ser Pro Leu Thr Thr Phe Gln Asp Gln Leu Asn Arg Arg Gly
                85                  90                  95
```

gag ttc atc cag gaa att agg aga cag ctg gaa gcc tgt caa aga gag 336
Glu Phe Ile Gln Glu Ile Arg Arg Gln Leu Glu Ala Cys Gln Arg Glu
100 105 110

aga gca ctt tcc gtg aag ttt gag gtc cag gct cca cgc tgg ggc aac 384
Arg Ala Leu Ser Val Lys Phe Glu Val Gln Ala Pro Arg Trp Gly Asn
115 120 125

ccc cgt gcg ctc agc ttc gta ctg agt tcg ctc cag ctc ggg gag ggg 432
Pro Arg Ala Leu Ser Phe Val Leu Ser Ser Leu Gln Leu Gly Glu Gly
130 135 140

gtg gag ttc gat gtg ctg cct gcc ttt gat gcc ctg ggt cag ttg act 480
Val Glu Phe Asp Val Leu Pro Ala Phe Asp Ala Leu Gly Gln Leu Thr
145 150 155 160

ggc agc tat aaa cct aac ccc caa atc tat gtc aag ctc atc gag gag 528
Gly Ser Tyr Lys Pro Asn Pro Gln Ile Tyr Val Lys Leu Ile Glu Glu
165 170 175

tgc acc gac ctg cag aaa gag ggc gag ttc tcc acc tgc ttc aca gaa 576
Cys Thr Asp Leu Gln Lys Glu Gly Glu Phe Ser Thr Cys Phe Thr Glu
180 185 190

cta cag aga gac ttc ctg aag cag cgc ccc acc aag ctc aag agc ctc 624
Leu Gln Arg Asp Phe Leu Lys Gln Arg Pro Thr Lys Leu Lys Ser Leu
195 200 205

atc cgc cta gtc aag cac tgg tac caa aat tgt aag aag aag ctt ggg 672
Ile Arg Leu Val Lys His Trp Tyr Gln Asn Cys Lys Lys Lys Leu Gly
210 215 220

aag ctg cca cct cag tat gcc ctg gag ctc ctg acg gtc tat gct tgg 720
Lys Leu Pro Pro Gln Tyr Ala Leu Glu Leu Leu Thr Val Tyr Ala Trp
225 230 235 240

gag cga ggg agc atg aaa aca cat ttc aac aca gcc caa gga ttt cgg 768
Glu Arg Gly Ser Met Lys Thr His Phe Asn Thr Ala Gln Gly Phe Arg
245 250 255

acg gtc ttg gaa tta gtc ata aac tac cag caa ctc tgc atc tac tgg 816
Thr Val Leu Glu Leu Val Ile Asn Tyr Gln Gln Leu Cys Ile Tyr Trp
260 265 270

aca aag tat tat gac ttt aaa aac ccc att att gaa aag tac ctg aga 864
Thr Lys Tyr Tyr Asp Phe Lys Asn Pro Ile Ile Glu Lys Tyr Leu Arg
275 280 285

agg cag ctc acg aaa ccc agg cct gtg atc ctg gac ccg gcg gac cct 912
Arg Gln Leu Thr Lys Pro Arg Pro Val Ile Leu Asp Pro Ala Asp Pro
290 295 300

aca gga aac ttg ggt ggt gga gac cca aag ggt tgg agg cag ctg gca 960
Thr Gly Asn Leu Gly Gly Gly Asp Pro Lys Gly Trp Arg Gln Leu Ala
305 310 315 320

caa gag gct gag gcc tgg ctg aat tac cca tgc ttt aag aat tgg gat 1008
Gln Glu Ala Glu Ala Trp Leu Asn Tyr Pro Cys Phe Lys Asn Trp Asp
325 330 335

ggg tcc cca gtg agc tcc tgg att ctg ctg gct gaa agc aac agt aca 1056

```
Gly Ser Pro Val Ser Ser Trp Ile Leu Leu Ala Glu Ser Asn Ser Thr
            340                 345                 350

gac gat gag acc gac gat ccc agg acg tat cag aaa tat ggt tac att      1104
Asp Asp Glu Thr Asp Asp Pro Arg Thr Tyr Gln Lys Tyr Gly Tyr Ile
        355                 360                 365

gga aca cat gag tac cct cat ttc tct cat aga ccc agc acg ctc cag      1152
Gly Thr His Glu Tyr Pro His Phe Ser His Arg Pro Ser Thr Leu Gln
    370                 375                 380

gca gca tcc acc cca cag gca gaa gag gac tgg acc tgc acc atc ctc      1200
Ala Ala Ser Thr Pro Gln Ala Glu Glu Asp Trp Thr Cys Thr Ile Leu
385                 390                 395                 400

tga atg c                                                            1207
    Met


<210>  4
<211>  400
<212>  PRT
<213>  Homo sapiens

<400>  4

Met Met Asp Leu Arg Asn Thr Pro Ala Lys Ser Leu Asp Lys Phe Ile
1               5                   10                  15


Glu Asp Tyr Leu Leu Pro Asp Thr Cys Phe Arg Met Gln Ile Asp His
            20                  25                  30


Ala Ile Asp Ile Ile Cys Gly Phe Leu Lys Glu Arg Cys Phe Arg Gly
        35                  40                  45


Ser Ser Tyr Pro Val Cys Val Ser Lys Val Val Lys Gly Gly Ser Ser
    50                  55                  60


Gly Lys Gly Thr Thr Leu Arg Gly Arg Ser Asp Ala Asp Leu Val Val
65                  70                  75                  80


Phe Leu Ser Pro Leu Thr Thr Phe Gln Asp Gln Leu Asn Arg Arg Gly
                85                  90                  95


Glu Phe Ile Gln Glu Ile Arg Arg Gln Leu Glu Ala Cys Gln Arg Glu
            100                 105                 110


Arg Ala Leu Ser Val Lys Phe Glu Val Gln Ala Pro Arg Trp Gly Asn
        115                 120                 125


Pro Arg Ala Leu Ser Phe Val Leu Ser Ser Leu Gln Leu Gly Glu Gly
    130                 135                 140
```

Val Glu Phe Asp Val Leu Pro Ala Phe Asp Ala Leu Gly Gln Leu Thr
145 150 155 160

Gly Ser Tyr Lys Pro Asn Pro Gln Ile Tyr Val Lys Leu Ile Glu Glu
165 170 175

Cys Thr Asp Leu Gln Lys Glu Gly Glu Phe Ser Thr Cys Phe Thr Glu
180 185 190

Leu Gln Arg Asp Phe Leu Lys Gln Arg Pro Thr Lys Leu Lys Ser Leu
195 200 205

Ile Arg Leu Val Lys His Trp Tyr Gln Asn Cys Lys Lys Lys Leu Gly
210 215 220

Lys Leu Pro Pro Gln Tyr Ala Leu Glu Leu Leu Thr Val Tyr Ala Trp
225 230 235 240

Glu Arg Gly Ser Met Lys Thr His Phe Asn Thr Ala Gln Gly Phe Arg
245 250 255

Thr Val Leu Glu Leu Val Ile Asn Tyr Gln Gln Leu Cys Ile Tyr Trp
260 265 270

Thr Lys Tyr Tyr Asp Phe Lys Asn Pro Ile Ile Glu Lys Tyr Leu Arg
275 280 285

Arg Gln Leu Thr Lys Pro Arg Pro Val Ile Leu Asp Pro Ala Asp Pro
290 295 300

Thr Gly Asn Leu Gly Gly Gly Asp Pro Lys Gly Trp Arg Gln Leu Ala
305 310 315 320

Gln Glu Ala Glu Ala Trp Leu Asn Tyr Pro Cys Phe Lys Asn Trp Asp
325 330 335

Gly Ser Pro Val Ser Ser Trp Ile Leu Leu Ala Glu Ser Asn Ser Thr
340 345 350

Asp Asp Glu Thr Asp Asp Pro Arg Thr Tyr Gln Lys Tyr Gly Tyr Ile
355 360 365

Gly Thr His Glu Tyr Pro His Phe Ser His Arg Pro Ser Thr Leu Gln
370 375 380

```
Ala Ala Ser Thr Pro Gln Ala Glu Glu Asp Trp Thr Cys Thr Ile Leu
385                 390                 395                 400
```

<210> 5
<211> 2064
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(2064)
<223>

<300>
<301> Hovnanian, A., et al.
<302> The human 2', 5'-oligoadenylate synthetase locus is comosed of three distinct genes
<303> Genomics
<304> 52
<305> 3
<306> 267-277
<307> 1998
<308> NCBI/NM_002535
<309> 2003-04-03

<300>
<301> Marie, I. and Hovanessian, A.G.
<302> The 69-kDa 2-5A synthetase is composed of two homologous and adjacent functional domains
<303> J. Biol. Chem.
<304> 267
<305> 14
<306> 9933-9939
<307> 1992
<308> NCBI/NM_002535
<309> 2003-04-03

<300>
<301> Marie, I., et al.
<302> Differential expression and distinct structure of 69- and 100-kDa forms of 2-5A synthetase
<303> J. Biol. Chem.
<304> 265
<305> 30
<306> 18601-18607
<307> 1990
<308> NCBI/NM_002535
<309> 2003-04-03

<300>
<301> Marie, I., et al.
<302> Preparation and characterization of polyclonal antibodies specific for the 69 and 100 k-dalton forms of human 2-5A synthetase
<303> Biochem. Biophys. Res. Commun.
<304> 160
<305> 2
<306> 580-587
<307> 1989

<308> NCBI/NM_002535
<309> 2003-04-03

<300>
<301> Hovanessian, A.G., et al.
<302> Characterization of 69- and 100-kDa forms of 2-5A-synthetase from interferon-treated human cells
<303> J. Biol. Chem.
<304> 263
<305> 10
<306> 4959
<307> 1988
<308> NCBI/NM_002535
<309> 2003-04-03

<300>
<301> Hovanessian, A.G., et al.
<302> Identification of 69-kd and 100-kd forms of 2-5A synthesase
<303> EMBO J.
<304> 6
<305> 5
<306> 1273-1280
<307> 1987
<308> NCBI/NM_002535
<309> 2003-04-03

<400> 5
atg gga aat ggg gag tcc cag ctg tcc tcg gtg cct gct cag aag ctg 48
Met Gly Asn Gly Glu Ser Gln Leu Ser Ser Val Pro Ala Gln Lys Leu
1 5 10 15

ggt tgg ttt atc cag gaa tac ctg aag ccc tac gaa gaa tgt cag aca 96
Gly Trp Phe Ile Gln Glu Tyr Leu Lys Pro Tyr Glu Glu Cys Gln Thr
20 25 30

ctg atc gac gag atg gtg aac acc atc tgt gac gtc tgc agg aac ccc 144
Leu Ile Asp Glu Met Val Asn Thr Ile Cys Asp Val Cys Arg Asn Pro
35 40 45

gaa cag ttc ccc ctg gtg cag gga gtg gcc ata ggt ggc tcc tat gga 192
Glu Gln Phe Pro Leu Val Gln Gly Val Ala Ile Gly Gly Ser Tyr Gly
50 55 60

cgg aaa aca gtc tta aga ggc aac tcc gat ggt acc ctt gtc ctt ttc 240
Arg Lys Thr Val Leu Arg Gly Asn Ser Asp Gly Thr Leu Val Leu Phe
65 70 75 80

ttc agt gac tta aaa caa ttc cag gat cag aag aga agc caa cgt gac 288
Phe Ser Asp Leu Lys Gln Phe Gln Asp Gln Lys Arg Ser Gln Arg Asp
85 90 95

atc ctc gat aaa act ggg gat aag ctg aag ttc tgt ctg ttc acg aag 336
Ile Leu Asp Lys Thr Gly Asp Lys Leu Lys Phe Cys Leu Phe Thr Lys
100 105 110

tgg ttg aaa aac aat ttc gag atc cag aag tcc ctt gat ggg tcc acc 384
Trp Leu Lys Asn Asn Phe Glu Ile Gln Lys Ser Leu Asp Gly Ser Thr
115 120 125

atc cag gtg ttc aca aaa aat cag aga atc tct ttc gag gtg ctg gcc 432
Ile Gln Val Phe Thr Lys Asn Gln Arg Ile Ser Phe Glu Val Leu Ala

```
    130                 135                 140

gcc ttc aac gct ctg agc tta aat gat aat ccc agc ccc tgg atc tat      480
Ala Phe Asn Ala Leu Ser Leu Asn Asp Asn Pro Ser Pro Trp Ile Tyr
145                 150                 155                 160

cga gag ctc aaa aga tcc ttg gat aag aca aat gcc agt cct ggt gag      528
Arg Glu Leu Lys Arg Ser Leu Asp Lys Thr Asn Ala Ser Pro Gly Glu
                165                 170                 175

ttt gca gtc tgc ttc act gaa ctc cag cag aag ttt ttt gac aac cgt      576
Phe Ala Val Cys Phe Thr Glu Leu Gln Gln Lys Phe Phe Asp Asn Arg
            180                 185                 190

cct gga aaa cta aag gat ttg atc ctc ttg ata aag cac tgg cat caa      624
Pro Gly Lys Leu Lys Asp Leu Ile Leu Leu Ile Lys His Trp His Gln
        195                 200                 205

cag tgc cag aaa aaa atc aag gat tta ccc tcg ctg tct ccg tat gcc      672
Gln Cys Gln Lys Lys Ile Lys Asp Leu Pro Ser Leu Ser Pro Tyr Ala
    210                 215                 220

ctg gag ctg ctt acg gtg tat gcc tgg gaa cag ggg tgc aga aaa gac      720
Leu Glu Leu Leu Thr Val Tyr Ala Trp Glu Gln Gly Cys Arg Lys Asp
225                 230                 235                 240

aac ttt gac att gct gaa ggc gtc aga acg gtt ctg gag ctg atc aaa      768
Asn Phe Asp Ile Ala Glu Gly Val Arg Thr Val Leu Glu Leu Ile Lys
                245                 250                 255

tgc cag gag aag ctg tgt atc tat tgg atg gtc aac tac aac ttt gaa      816
Cys Gln Glu Lys Leu Cys Ile Tyr Trp Met Val Asn Tyr Asn Phe Glu
            260                 265                 270

gat gag acc atc agg aac atc ctg ctg cac cag ctc caa tca gcg agg      864
Asp Glu Thr Ile Arg Asn Ile Leu Leu His Gln Leu Gln Ser Ala Arg
        275                 280                 285

cca gta atc ttg gat cca gtt gac cca acc aat aat gtg agt gga gat      912
Pro Val Ile Leu Asp Pro Val Asp Pro Thr Asn Asn Val Ser Gly Asp
    290                 295                 300

aaa ata tgc tgg caa tgg ctg aaa aaa gaa gct caa acc tgg ttg act      960
Lys Ile Cys Trp Gln Trp Leu Lys Lys Glu Ala Gln Thr Trp Leu Thr
305                 310                 315                 320

tct ccc aac ctg gat aat gag tta cct gca cca tct tgg aat gtc ctg     1008
Ser Pro Asn Leu Asp Asn Glu Leu Pro Ala Pro Ser Trp Asn Val Leu
                325                 330                 335

cct gca cca ctc ttc acg acc cca ggc cac ctt ctg gat aag ttc atc     1056
Pro Ala Pro Leu Phe Thr Thr Pro Gly His Leu Leu Asp Lys Phe Ile
            340                 345                 350

aag gag ttt ctc cag ccc aac aaa tgc ttc cta gag cag att gac agt     1104
Lys Glu Phe Leu Gln Pro Asn Lys Cys Phe Leu Glu Gln Ile Asp Ser
        355                 360                 365

gct gtt aac atc atc cgt aca ttc ctt aaa gaa aac tgc ttc cga caa     1152
Ala Val Asn Ile Ile Arg Thr Phe Leu Lys Glu Asn Cys Phe Arg Gln
    370                 375                 380
```

```
tca aca gcc aag atc cag att gtc cgg gga gga tca acc gcc aaa ggc      1200
Ser Thr Ala Lys Ile Gln Ile Val Arg Gly Gly Ser Thr Ala Lys Gly
385                 390                 395                 400

aca gct ctg aag act ggc tct gat gcc gat ctc gtc gtg ttc cat aac      1248
Thr Ala Leu Lys Thr Gly Ser Asp Ala Asp Leu Val Val Phe His Asn
                405                 410                 415

tca ctt aaa agc tac acc tcc caa aaa aac gag cgg cac aaa atc gtc      1296
Ser Leu Lys Ser Tyr Thr Ser Gln Lys Asn Glu Arg His Lys Ile Val
            420                 425                 430

aag gaa atc cat gaa cag ctg aaa gcc ttt tgg agg gag aag gag gag      1344
Lys Glu Ile His Glu Gln Leu Lys Ala Phe Trp Arg Glu Lys Glu Glu
        435                 440                 445

gag ctt gaa gtc agc ttt gag cct ccc aag tgg aag gct ccc agg gtg      1392
Glu Leu Glu Val Ser Phe Glu Pro Pro Lys Trp Lys Ala Pro Arg Val
    450                 455                 460

ctg agc ttc tct ctg aaa tcc aaa gtc ctc aac gaa agt gtc agc ttt      1440
Leu Ser Phe Ser Leu Lys Ser Lys Val Leu Asn Glu Ser Val Ser Phe
465                 470                 475                 480

gat gtg ctt cct gcc ttt aat gca ctg ggt cag ctg agt tct ggc tcc      1488
Asp Val Leu Pro Ala Phe Asn Ala Leu Gly Gln Leu Ser Ser Gly Ser
                485                 490                 495

aca ccc agc ccc gag gtt tat gca ggg ctc att gat ctg tat aaa tcc      1536
Thr Pro Ser Pro Glu Val Tyr Ala Gly Leu Ile Asp Leu Tyr Lys Ser
            500                 505                 510

tcg gac ctc ccg gga gga gag ttt tct acc tgt ttc aca gtc ctg cag      1584
Ser Asp Leu Pro Gly Gly Glu Phe Ser Thr Cys Phe Thr Val Leu Gln
        515                 520                 525

cga aac ttc att cgc tcc cgg ccc acc aaa cta aag gat tta att cgc      1632
Arg Asn Phe Ile Arg Ser Arg Pro Thr Lys Leu Lys Asp Leu Ile Arg
    530                 535                 540

ctg gtg aag cac tgg tac aaa gag tgt gaa agg aaa ctg aag cca aag      1680
Leu Val Lys His Trp Tyr Lys Glu Cys Glu Arg Lys Leu Lys Pro Lys
545                 550                 555                 560

ggg tct ttg ccc cca aag tat gcc ttg gag ctg ctc acc atc tat gcc      1728
Gly Ser Leu Pro Pro Lys Tyr Ala Leu Glu Leu Leu Thr Ile Tyr Ala
                565                 570                 575

tgg gag cag ggg agt gga gtg ccg gat ttt gac act gca gaa ggt ttc      1776
Trp Glu Gln Gly Ser Gly Val Pro Asp Phe Asp Thr Ala Glu Gly Phe
            580                 585                 590

cgg aca gtc ctg gag ctg gtc aca caa tat cag cag ctc ggc atc ttc      1824
Arg Thr Val Leu Glu Leu Val Thr Gln Tyr Gln Gln Leu Gly Ile Phe
        595                 600                 605

tgg aag gtc aat tac aac ttt gaa gat gag acc gtg agg aag ttt cta      1872
Trp Lys Val Asn Tyr Asn Phe Glu Asp Glu Thr Val Arg Lys Phe Leu
    610                 615                 620
```

ctg agc cag ttg cag aaa acc agg cct gtg atc ttg gac cca ggc gaa 1920
Leu Ser Gln Leu Gln Lys Thr Arg Pro Val Ile Leu Asp Pro Gly Glu
625 630 635 640

ccc aca ggt gac gtg ggt gga ggg gac cgt tgg tgt tgg cat ctt ctg 1968
Pro Thr Gly Asp Val Gly Gly Gly Asp Arg Trp Cys Trp His Leu Leu
645 650 655

gac aaa gaa gca aag gtt agg tta tcc tct ccc tgc ttc aag gat ggg 2016
Asp Lys Glu Ala Lys Val Arg Leu Ser Ser Pro Cys Phe Lys Asp Gly
660 665 670

act gga aac cca ata cca cct tgg aaa gtg ccg gta aaa gtc atc taa 2064
Thr Gly Asn Pro Ile Pro Pro Trp Lys Val Pro Val Lys Val Ile
675 680 685

<210> 6
<211> 687
<212> PRT
<213> Homo sapiens

<400> 6

Met Gly Asn Gly Glu Ser Gln Leu Ser Ser Val Pro Ala Gln Lys Leu
1 5 10 15

Gly Trp Phe Ile Gln Glu Tyr Leu Lys Pro Tyr Glu Glu Cys Gln Thr
20 25 30

Leu Ile Asp Glu Met Val Asn Thr Ile Cys Asp Val Cys Arg Asn Pro
35 40 45

Glu Gln Phe Pro Leu Val Gln Gly Val Ala Ile Gly Gly Ser Tyr Gly
50 55 60

Arg Lys Thr Val Leu Arg Gly Asn Ser Asp Gly Thr Leu Val Leu Phe
65 70 75 80

Phe Ser Asp Leu Lys Gln Phe Gln Asp Gln Lys Arg Ser Gln Arg Asp
85 90 95

Ile Leu Asp Lys Thr Gly Asp Lys Leu Lys Phe Cys Leu Phe Thr Lys
100 105 110

Trp Leu Lys Asn Asn Phe Glu Ile Gln Lys Ser Leu Asp Gly Ser Thr
115 120 125

Ile Gln Val Phe Thr Lys Asn Gln Arg Ile Ser Phe Glu Val Leu Ala
130 135 140

Ala Phe Asn Ala Leu Ser Leu Asn Asp Asn Pro Ser Pro Trp Ile Tyr

145 150 155 160

Arg Glu Leu Lys Arg Ser Leu Asp Lys Thr Asn Ala Ser Pro Gly Glu
165 170 175

Phe Ala Val Cys Phe Thr Glu Leu Gln Gln Lys Phe Phe Asp Asn Arg
180 185 190

Pro Gly Lys Leu Lys Asp Leu Ile Leu Leu Ile Lys His Trp His Gln
195 200 205

Gln Cys Gln Lys Lys Ile Lys Asp Leu Pro Ser Leu Ser Pro Tyr Ala
210 215 220

Leu Glu Leu Leu Thr Val Tyr Ala Trp Glu Gln Gly Cys Arg Lys Asp
225 230 235 240

Asn Phe Asp Ile Ala Glu Gly Val Arg Thr Val Leu Glu Leu Ile Lys
245 250 255

Cys Gln Glu Lys Leu Cys Ile Tyr Trp Met Val Asn Tyr Asn Phe Glu
260 265 270

Asp Glu Thr Ile Arg Asn Ile Leu Leu His Gln Leu Gln Ser Ala Arg
275 280 285

Pro Val Ile Leu Asp Pro Val Asp Pro Thr Asn Asn Val Ser Gly Asp
290 295 300

Lys Ile Cys Trp Gln Trp Leu Lys Lys Glu Ala Gln Thr Trp Leu Thr
305 310 315 320

Ser Pro Asn Leu Asp Asn Glu Leu Pro Ala Pro Ser Trp Asn Val Leu
325 330 335

Pro Ala Pro Leu Phe Thr Thr Pro Gly His Leu Leu Asp Lys Phe Ile
340 345 350

Lys Glu Phe Leu Gln Pro Asn Lys Cys Phe Leu Glu Gln Ile Asp Ser
355 360 365

Ala Val Asn Ile Ile Arg Thr Phe Leu Lys Glu Asn Cys Phe Arg Gln
370 375 380

Ser Thr Ala Lys Ile Gln Ile Val Arg Gly Gly Ser Thr Ala Lys Gly
385 390 395 400

```
Thr Ala Leu Lys Thr Gly Ser Asp Ala Asp Leu Val Val Phe His Asn
                405                 410                 415

Ser Leu Lys Ser Tyr Thr Ser Gln Lys Asn Glu Arg His Lys Ile Val
            420                 425                 430

Lys Glu Ile His Glu Gln Leu Lys Ala Phe Trp Arg Glu Lys Glu Glu
        435                 440                 445

Glu Leu Glu Val Ser Phe Glu Pro Pro Lys Trp Lys Ala Pro Arg Val
    450                 455                 460

Leu Ser Phe Ser Leu Lys Ser Lys Val Leu Asn Glu Ser Val Ser Phe
465                 470                 475                 480

Asp Val Leu Pro Ala Phe Asn Ala Leu Gly Gln Leu Ser Ser Gly Ser
                485                 490                 495

Thr Pro Ser Pro Glu Val Tyr Ala Gly Leu Ile Asp Leu Tyr Lys Ser
            500                 505                 510

Ser Asp Leu Pro Gly Gly Glu Phe Ser Thr Cys Phe Thr Val Leu Gln
        515                 520                 525

Arg Asn Phe Ile Arg Ser Arg Pro Thr Lys Leu Lys Asp Leu Ile Arg
    530                 535                 540

Leu Val Lys His Trp Tyr Lys Glu Cys Glu Arg Lys Leu Lys Pro Lys
545                 550                 555                 560

Gly Ser Leu Pro Pro Lys Tyr Ala Leu Glu Leu Leu Thr Ile Tyr Ala
                565                 570                 575

Trp Glu Gln Gly Ser Gly Val Pro Asp Phe Asp Thr Ala Glu Gly Phe
            580                 585                 590

Arg Thr Val Leu Glu Leu Val Thr Gln Tyr Gln Gln Leu Gly Ile Phe
        595                 600                 605

Trp Lys Val Asn Tyr Asn Phe Glu Asp Glu Thr Val Arg Lys Phe Leu
    610                 615                 620

Leu Ser Gln Leu Gln Lys Thr Arg Pro Val Ile Leu Asp Pro Gly Glu
625                 630                 635                 640
```

```
Pro Thr Gly Asp Val Gly Gly Gly Asp Arg Trp Cys Trp His Leu Leu
                645                 650                 655

Asp Lys Glu Ala Lys Val Arg Leu Ser Ser Pro Cys Phe Lys Asp Gly
            660                 665                 670

Thr Gly Asn Pro Ile Pro Pro Trp Lys Val Pro Val Lys Val Ile
        675                 680                 685
```

<210> 7
<211> 2186
<212> DNA
<213> Homo sapiens

<300>
<301> Marie, I. and Hovanessian, A.G.
<302> The 69-kDa 2-5A synthetase is composed of two homologous and adjacent functional domains
<303> J. Biol. Chem.
<304> 267
<305> 14
<306> 9933-9939
<307> 1992
<308> (unknown)
<309> 2003-04-03

<400> 7

```
atgggaaatg gggagtccca gctgtcctcg gtgcctgctc agaagctggg ttggtttatc     60

caggaatacc tgaagcccta cgaagaatgt cagacactga tcgacgagat ggtgaacacc    120

atctgtgacg tctgcaggaa ccccgaacag ttcccccctgg tgcagggagt ggccataggt    180

ggctcctatg gacggaaaac agtcttaaga ggcaactccg atggtaccct tgtccttttc    240

ttcagtgact taaaacaatt ccaggatcag aagagaagcc aacgtgacat cctcgataaa    300

actggggata agctgaagtt ctgtctgttc acgaagtggt tgaaaaacaa tttcgagatc    360

cagaagtccc ttgatgggtc caccatccag gtgttcacaa aaaatcagag aatctctttc    420

gaggtgctgg ccgccttcaa cgctctgagc ttaaatgata atcccagccc ctggatctat    480

cgagagctca aaagatcctt ggataagaca aatgccagtc ctggtgagtt tgcagtctgc    540

ttcactgaac tccagcagaa gtttttttgac aaccgtcctg gaaaactaaa ggatttgatc    600

ctcttgataa agcactggca tcaacagtgc cagaaaaaaa tcaaggattt accctcgctg    660

tctccgtatg ccctggagct gcttacggtg tatgcctggg aacaggggtg cagaaaagac    720

aactttgaca ttgctgaagg cgtcagaacg gttctggagc tgatcaaatg ccaggagaag    780

ctgtgtatct attggatggt caactacaac tttgaagatg agaccatcag gaacatcctg    840

ctgcaccagc tccaatcagc gaggccagta atcttggatc cagttgaccc aaccaataat    900
```

gtgagtggag ataaaatatg ctggcaatgg ctgaaaaaag aagctcaaac ctggttgact 960

tctcccaacc tggataatga gttacctgca ccatcttgga atgtcctgcc tgcaccactc 1020

ttcacgaccc caggccacct tctggataag ttcatcaagg agtttctcca gcccaacaaa 1080

tgcttcctag agcagattga cagtgctgtt aacatcatcc gtacattcct taaagaaaac 1140

tgcttccgac aatcaacagc caagatccag attgtccggg gaggatcaac cgccaaaggc 1200

acagctctga agactggctc tgatgccgat ctcgtcgtgt tccataactc acttaaaagc 1260

tacacctccc aaaaaaacga gcggcacaaa atcgtcaagg aaatccatga acagctgaaa 1320

gccttttgga gggagaagga ggaggagctt gaagtcagct ttgagcctcc caagtggaag 1380

gctcccaggg tgctgagctt ctctctgaaa tccaaagtcc tcaacgaaag tgtcagcttt 1440

gatgtgcttc ctgcctttaa tgcactgggt cagctgagtt ctggctccac acccagcccc 1500

gaggtttatg cagggctcat tgatctgtat aaatcctcgg acctcccggg aggagagttt 1560

tctacctgtt tcacagtcct gcagcgaaac ttcattcgct cccggcccac caaactaaag 1620

gatttaattc gcctggtgaa gcactggtac aaagagtgtg aaaggaaact gaagccaaag 1680

gggtctttgc cccccaaagta tgccttggag ctgctcacca tctatgcctg ggagcagggg 1740

agtggagtgc cggattttga cactgcagaa ggtttccgga cagtcctgga gctggtcaca 1800

caatatcagc agctcggcat cttctggaag gtcaattaca actttgaaga tgagaccgtg 1860

aggaagtttc tactgagcca gttgcagaaa accaggcctg tgatcttgga cccaggcgaa 1920

cccacaggtg acgtgggtgg aggggaccgt tggtgttggc atcttctgga caaagaagca 1980

aaggttaggt tatcctctcc ctgcttcaag gatgggactg gaaacccaat accaccttgg 2040

aaagtgccga caatgcagac accaggaagt tgtggagcta ggattccatc ctattgtcaa 2100

tgagatgttg tcatccagaa gccatagaat cctgaataat aattctaaaa gaaacttctg 2160

gagatcatct ggcaatcgct tttaaa 2186

<210> 8
<211> 727
<212> PRT
<213> Homo sapiens

<400> 8

Met Gly Asn Gly Glu Ser Gln Leu Ser Ser Val Pro Ala Gln Lys Leu
1 5 10 15

Gly Trp Phe Ile Gln Glu Tyr Leu Lys Pro Tyr Glu Glu Cys Gln Thr
20 25 30

Leu Ile Asp Glu Met Val Asn Thr Ile Cys Asp Val Cys Arg Asn Pro

35 40 45

Glu Gln Phe Pro Leu Val Gln Gly Val Ala Ile Gly Gly Ser Tyr Gly
50 55 60

Arg Lys Thr Val Leu Arg Gly Asn Ser Asp Gly Thr Leu Val Leu Phe
65 70 75 80

Phe Ser Asp Leu Lys Gln Phe Gln Asp Gln Lys Arg Ser Gln Arg Asp
85 90 95

Ile Leu Asp Lys Thr Gly Asp Lys Leu Lys Phe Cys Leu Phe Thr Lys
100 105 110

Trp Leu Lys Asn Asn Phe Glu Ile Gln Lys Ser Leu Asp Gly Ser Thr
115 120 125

Ile Gln Val Phe Thr Lys Asn Gln Arg Ile Ser Phe Glu Val Leu Ala
130 135 140

Ala Phe Asn Ala Leu Ser Leu Asn Asp Asn Pro Ser Pro Trp Ile Tyr
145 150 155 160

Arg Glu Leu Lys Arg Ser Leu Asp Lys Thr Asn Ala Ser Pro Gly Glu
165 170 175

Phe Ala Val Cys Phe Thr Glu Leu Gln Gln Lys Phe Phe Asp Asn Arg
180 185 190

Pro Gly Lys Leu Lys Asp Leu Ile Leu Leu Ile Lys His Trp His Gln
195 200 205

Gln Cys Gln Lys Lys Ile Lys Asp Leu Pro Ser Leu Ser Pro Tyr Ala
210 215 220

Leu Glu Leu Leu Thr Val Tyr Ala Trp Glu Gln Gly Cys Arg Lys Asp
225 230 235 240

Asn Phe Asp Ile Ala Glu Gly Val Arg Thr Val Leu Glu Leu Ile Lys
245 250 255

Cys Gln Glu Lys Leu Cys Ile Tyr Trp Met Val Asn Tyr Asn Phe Glu
260 265 270

Asp Glu Thr Ile Arg Asn Ile Leu Leu His Gln Leu Gln Ser Ala Arg
275 280 285

Pro Val Ile Leu Asp Pro Val Asp Pro Thr Asn Asn Val Ser Gly Asp
290 295 300

Lys Ile Cys Trp Gln Trp Leu Lys Lys Glu Ala Gln Thr Trp Leu Thr
305 310 315 320

Ser Pro Asn Leu Asp Asn Glu Leu Pro Ala Pro Ser Trp Asn Val Leu
325 330 335

Pro Ala Pro Leu Phe Thr Thr Pro Gly His Leu Leu Asp Lys Phe Ile
340 345 350

Lys Glu Phe Leu Gln Pro Asn Lys Cys Phe Leu Glu Gln Ile Asp Ser
355 360 365

Ala Val Asn Ile Ile Arg Thr Phe Leu Lys Glu Asn Cys Phe Arg Gln
370 375 380

Ser Thr Ala Lys Ile Gln Ile Val Arg Gly Gly Ser Thr Ala Lys Gly
385 390 395 400

Thr Ala Leu Lys Thr Gly Ser Asp Ala Asp Leu Val Val Phe His Asn
405 410 415

Ser Leu Lys Ser Tyr Thr Ser Gln Lys Asn Glu Arg His Lys Ile Val
420 425 430

Lys Glu Ile His Glu Gln Leu Lys Ala Phe Trp Arg Glu Lys Glu Glu
435 440 445

Glu Leu Glu Val Ser Phe Glu Pro Pro Lys Trp Lys Ala Pro Arg Val
450 455 460

Leu Ser Phe Ser Leu Lys Ser Lys Val Leu Asn Glu Ser Val Ser Phe
465 470 475 480

Asp Val Leu Pro Ala Phe Asn Ala Leu Gly Gln Leu Ser Ser Gly Ser
485 490 495

Thr Pro Ser Pro Glu Val Tyr Ala Gly Leu Ile Asp Leu Tyr Lys Ser
500 505 510

Ser Asp Leu Pro Gly Gly Glu Phe Ser Thr Cys Phe Thr Val Leu Gln
515 520 525

Arg Asn Phe Ile Arg Ser Arg Pro Thr Lys Leu Lys Asp Leu Ile Arg
530 535 540

Leu Val Lys His Trp Tyr Lys Glu Cys Glu Arg Lys Leu Lys Pro Lys
545 550 555 560

Gly Ser Leu Pro Pro Lys Tyr Ala Leu Glu Leu Leu Thr Ile Tyr Ala
565 570 575

Trp Glu Gln Gly Ser Gly Val Pro Asp Phe Asp Thr Ala Glu Gly Phe
580 585 590

Arg Thr Val Leu Glu Leu Val Thr Gln Tyr Gln Gln Leu Gly Ile Phe
595 600 605

Trp Lys Val Asn Tyr Asn Phe Glu Asp Glu Thr Val Arg Lys Phe Leu
610 615 620

Leu Ser Gln Leu Gln Lys Thr Arg Pro Val Ile Leu Asp Pro Gly Glu
625 630 635 640

Pro Thr Gly Asp Val Gly Gly Gly Asp Arg Trp Cys Trp His Leu Leu
645 650 655

Asp Lys Glu Ala Lys Val Arg Leu Ser Ser Pro Cys Phe Lys Asp Gly
660 665 670

Thr Gly Asn Pro Ile Pro Pro Trp Lys Val Pro Thr Met Gln Thr Pro
675 680 685

Gly Ser Cys Gly Ala Arg Ile His Pro Ile Val Asn Glu Met Phe Ser
690 695 700

Ser Arg Ser His Arg Ile Leu Asn Asn Asn Ser Lys Arg Asn Phe Thr
705 710 715 720

Arg Ser Ser Gly Asn Arg Phe
725

<210> 9
<211> 3264
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(3264)

<223>

<300>
<301> Rebouillat, D., et al.
<302> The 100-kDa 2',5'-oligoadenylate synthetase catalyzing preferentially the synthesis of dimeric pppA2'p5'A molecules
<303> J. Biol. Chem.
<304> 274
<305> 3
<306> 1557-1565
<307> 1999
<308> NCBI/AF_063613
<309> 1999-05-04

<300>
<301> Rebouillat, D. and Hovanessian, A.G.
<302> Direct Submission
<303> Submitted (07-May-1998) Dept.of Aids and Retroviruses, Institut Pasteur
<304> 0
<305> 0
<306> 0
<307> 1998
<308> NCBI/AF_063613
<309> 1999-05-04

<400> 9
atg gac ttg tac agc acc ccg gcc gct gcg ctg gac agg ttc gtg gcc 48
Met Asp Leu Tyr Ser Thr Pro Ala Ala Ala Leu Asp Arg Phe Val Ala
1   5   10   15

aga agg ctg cag ccg cgg aag gag ttc gta gag aag gcg cgg cgc gct 96
Arg Arg Leu Gln Pro Arg Lys Glu Phe Val Glu Lys Ala Arg Arg Ala
            20   25   30

ctg ggc gcc ctg gcc gct gcc ctg agg gag cgc ggg ggc cgc ctc ggt 144
Leu Gly Ala Leu Ala Ala Ala Leu Arg Glu Arg Gly Gly Arg Leu Gly
        35   40   45

gct gct gcc ccg cgg gtg ctg aaa act gtc aag gga ggc tcc tcg ggc 192
Ala Ala Ala Pro Arg Val Leu Lys Thr Val Lys Gly Gly Ser Ser Gly
    50   55   60

cgg ggc aca gct ctc aag ggt ggc tgt gat tct gaa ctt gtc atc ttc 240
Arg Gly Thr Ala Leu Lys Gly Gly Cys Asp Ser Glu Leu Val Ile Phe
65   70   75   80

ctc gac tgc ttc aag agc tat gtg gac cag agg gcc cgc cgt gca gag 288
Leu Asp Cys Phe Lys Ser Tyr Val Asp Gln Arg Ala Arg Arg Ala Glu
                85   90   95

atc ctc agt gag atg cgg gca tcg ctg gaa tcc tgg tgg cag aac cca 336
Ile Leu Ser Glu Met Arg Ala Ser Leu Glu Ser Trp Trp Gln Asn Pro
            100   105   110

gtc cct ggt ctg aga ctc acg ttt cct gag cag agc gtg cct ggg gcc 384
Val Pro Gly Leu Arg Leu Thr Phe Pro Glu Gln Ser Val Pro Gly Ala
        115   120   125

ctg cag ttc cgc ctg aca tcc gta gat ctt gag gac tgg atg gat gtt 432

Leu Gln Phe Arg Leu Thr Ser Val Asp Leu Glu Asp Trp Met Asp Val
130 135 140

agc ctg gtg cct gcc ttc aat gtc ctg ggt cag gcc ggc tcc gcg gtc 480
Ser Leu Val Pro Ala Phe Asn Val Leu Gly Gln Ala Gly Ser Ala Val
145 150 155 160

aaa ccc aag cca caa gtc tac tct acc ctc ctc aac agt ggc tgc caa 528
Lys Pro Lys Pro Gln Val Tyr Ser Thr Leu Leu Asn Ser Gly Cys Gln
165 170 175

ggg ggc gag cat gcg gcc tgc ttc aca gag ctg cgg agg aac ttt gtg 576
Gly Gly Glu His Ala Ala Cys Phe Thr Glu Leu Arg Arg Asn Phe Val
180 185 190

aac att cgc cca gcc aag ttg aag aac cta atc ttg ctg gtg aag cac 624
Asn Ile Arg Pro Ala Lys Leu Lys Asn Leu Ile Leu Leu Val Lys His
195 200 205

tgg tac cac cag gtg tgc cta cag ggg ttg tgg aag gag acg ctg ccc 672
Trp Tyr His Gln Val Cys Leu Gln Gly Leu Trp Lys Glu Thr Leu Pro
210 215 220

ccg gtc tat gcc ctg gaa ttg ctg acc atc ttc gcc tgg gag cag ggc 720
Pro Val Tyr Ala Leu Glu Leu Leu Thr Ile Phe Ala Trp Glu Gln Gly
225 230 235 240

tgt aag aag gat gct ttc agc cta ggc gaa ggc ctc cga act gtc ctg 768
Cys Lys Lys Asp Ala Phe Ser Leu Gly Glu Gly Leu Arg Thr Val Leu
245 250 255

ggc ctg atc caa cag cat cag cac ctg tgt gtt ttc tgg act gtc aac 816
Gly Leu Ile Gln Gln His Gln His Leu Cys Val Phe Trp Thr Val Asn
260 265 270

tat ggc ttc gag gac cct gca gtt ggg cag ttc ttg cag cgg cac gtt 864
Tyr Gly Phe Glu Asp Pro Ala Val Gly Gln Phe Leu Gln Arg His Val
275 280 285

aag aga ccc agg cct gtg atc ctg gac cca gct gac ccc aca tgg gac 912
Lys Arg Pro Arg Pro Val Ile Leu Asp Pro Ala Asp Pro Thr Trp Asp
290 295 300

ctg ggg aat ggg gca gcc tgg cac tgg gat ttg cat gcc cag gag gca 960
Leu Gly Asn Gly Ala Ala Trp His Trp Asp Leu His Ala Gln Glu Ala
305 310 315 320

gca tcc tgc tat gac cac cca tgc ttt ctg agg ggg atg ggg gac cca 1008
Ala Ser Cys Tyr Asp His Pro Cys Phe Leu Arg Gly Met Gly Asp Pro
325 330 335

gtg cag tct tgg aag ggg ccg ggc ctt cca cgt gct gga tgc tca ggt 1056
Val Gln Ser Trp Lys Gly Pro Gly Leu Pro Arg Ala Gly Cys Ser Gly
340 345 350

ttg ggc cac ccc atc cag cta gac cct aac cag aag acc cct gaa aac 1104
Leu Gly His Pro Ile Gln Leu Asp Pro Asn Gln Lys Thr Pro Glu Asn
355 360 365

agc aag agc ctc aat gct gtg tac cca aga gca ggg agc aaa cct ccc 1152
Ser Lys Ser Leu Asn Ala Val Tyr Pro Arg Ala Gly Ser Lys Pro Pro

```
    370                 375                 380

tca tgc cca gct cct ggc ccc act gcg gag cca gca tcg tac ccc tct      1200
Ser Cys Pro Ala Pro Gly Pro Thr Ala Glu Pro Ala Ser Tyr Pro Ser
385                 390                 395                 400

gtg ccg gga atg gcc ttg gac ctg tct cag atc ccc acc aag gag ctg      1248
Val Pro Gly Met Ala Leu Asp Leu Ser Gln Ile Pro Thr Lys Glu Leu
                405                 410                 415

gac cgc ttc atc cag gac cac ctg aag ccg agc ccc cag ttc cag gag      1296
Asp Arg Phe Ile Gln Asp His Leu Lys Pro Ser Pro Gln Phe Gln Glu
            420                 425                 430

cag gtg aaa aag gcc atc gac atc atc ttg cgc tgc ctc cat gag aac      1344
Gln Val Lys Lys Ala Ile Asp Ile Ile Leu Arg Cys Leu His Glu Asn
        435                 440                 445

tgt gtt cac aag gcc tca aga gtc agt aaa ggg ggc tca ttt ggc cgg      1392
Cys Val His Lys Ala Ser Arg Val Ser Lys Gly Gly Ser Phe Gly Arg
    450                 455                 460

ggc aca gac cta agg gat ggc tgt gat gtt gaa ctc atc atc ttc ctc      1440
Gly Thr Asp Leu Arg Asp Gly Cys Asp Val Glu Leu Ile Ile Phe Leu
465                 470                 475                 480

aac tgc ttc acg gac tac aag gac cag ggg ccc cgc cgc gca gag atc      1488
Asn Cys Phe Thr Asp Tyr Lys Asp Gln Gly Pro Arg Arg Ala Glu Ile
                485                 490                 495

ctt gat gag atg cga gcg cac gta gaa tcc tgg tgg cag gac cag gtg      1536
Leu Asp Glu Met Arg Ala His Val Glu Ser Trp Trp Gln Asp Gln Val
            500                 505                 510

ccc agc ctg agc ctt cag ttt cct gag cag aat gtg cct gag gct ctg      1584
Pro Ser Leu Ser Leu Gln Phe Pro Glu Gln Asn Val Pro Glu Ala Leu
        515                 520                 525

cag ttc cag ctg gtg tcc aca gcc ctg aag agc tgg acg gat gtt agc      1632
Gln Phe Gln Leu Val Ser Thr Ala Leu Lys Ser Trp Thr Asp Val Ser
    530                 535                 540

ctg ctg cct gcc ttc gat gct gtg ggg cag ctc agt tct ggc acc aaa      1680
Leu Leu Pro Ala Phe Asp Ala Val Gly Gln Leu Ser Ser Gly Thr Lys
545                 550                 555                 560

cca aat ccc cag gtc tac tcg agg ctc ctc acc agt ggc tgc cag gag      1728
Pro Asn Pro Gln Val Tyr Ser Arg Leu Leu Thr Ser Gly Cys Gln Glu
                565                 570                 575

ggc gag cat aag gcc tgc ttc gca gag ctg cgg agg aac ttc atg aac      1776
Gly Glu His Lys Ala Cys Phe Ala Glu Leu Arg Arg Asn Phe Met Asn
            580                 585                 590

att cgc cct gtc aag ctg aag aac ctg att ctg ctg gtg aag cac tgg      1824
Ile Arg Pro Val Lys Leu Lys Asn Leu Ile Leu Leu Val Lys His Trp
        595                 600                 605

tac cgc cag gtt gcg gct cag aac aaa gga aaa gga cca gcc cct gcc      1872
Tyr Arg Gln Val Ala Ala Gln Asn Lys Gly Lys Gly Pro Ala Pro Ala
    610                 615                 620
```

```
tct ctg ccc cca gcc tat gcc ctg gag ctc ctc acc atc ttt gcc tgg      1920
Ser Leu Pro Pro Ala Tyr Ala Leu Glu Leu Leu Thr Ile Phe Ala Trp
625                 630                 635                 640

gag cag ggc tgc agg cag gat tgt ttc aac atg gcc caa ggc ttc cgg      1968
Glu Gln Gly Cys Arg Gln Asp Cys Phe Asn Met Ala Gln Gly Phe Arg
                645                 650                 655

acg gtg ctg ggg ctc gtg caa cag cat cag cag ctc tgt gtc tac tgg      2016
Thr Val Leu Gly Leu Val Gln Gln His Gln Gln Leu Cys Val Tyr Trp
            660                 665                 670

acg gtc aac tat agc act gag gac cca gcc atg aga atg cac ctt ctt      2064
Thr Val Asn Tyr Ser Thr Glu Asp Pro Ala Met Arg Met His Leu Leu
        675                 680                 685

ggc cag ctt cga aaa ccc aga ccc ctg gtc ctg gac ccc gct gat ccc      2112
Gly Gln Leu Arg Lys Pro Arg Pro Leu Val Leu Asp Pro Ala Asp Pro
    690                 695                 700

acc tgg aac gtg ggc cac ggt agc tgg gag ctg ttg gcc cag gaa gca      2160
Thr Trp Asn Val Gly His Gly Ser Trp Glu Leu Leu Ala Gln Glu Ala
705                 710                 715                 720

gca gcg ctg ggg atg cag gcc tgc ttt ctg agt aga gac ggg aca tct      2208
Ala Ala Leu Gly Met Gln Ala Cys Phe Leu Ser Arg Asp Gly Thr Ser
                725                 730                 735

gtg cag ccc tgg gat gtg atg cca gcc ctc ctt tac caa acc cca gct      2256
Val Gln Pro Trp Asp Val Met Pro Ala Leu Leu Tyr Gln Thr Pro Ala
            740                 745                 750

ggg gac ctt gac aag ttc atc agt gaa ttt ctc cag ccc aac cgc cag      2304
Gly Asp Leu Asp Lys Phe Ile Ser Glu Phe Leu Gln Pro Asn Arg Gln
        755                 760                 765

ttc ctg gcc cag gtg aac aag gcc gtt gat acc atc tgt tca ttt ttg      2352
Phe Leu Ala Gln Val Asn Lys Ala Val Asp Thr Ile Cys Ser Phe Leu
    770                 775                 780

aag gaa aac tgc ttc cgg aat tct ccc atc aaa gtg atc aag gtg gtc      2400
Lys Glu Asn Cys Phe Arg Asn Ser Pro Ile Lys Val Ile Lys Val Val
785                 790                 795                 800

aag ggt ggc tct tca gcc aaa ggc aca gct ctg cga ggc cgc tca gat      2448
Lys Gly Gly Ser Ser Ala Lys Gly Thr Ala Leu Arg Gly Arg Ser Asp
                805                 810                 815

gcc gac ctc gtg gtg ttc ctc agc tgc ttc agc cag ttc act gag cag      2496
Ala Asp Leu Val Val Phe Leu Ser Cys Phe Ser Gln Phe Thr Glu Gln
            820                 825                 830

ggc aac aag cgg gcc gag atc atc tcc gag atc cga gcc cag ctg gag      2544
Gly Asn Lys Arg Ala Glu Ile Ile Ser Glu Ile Arg Ala Gln Leu Glu
        835                 840                 845

gca tgt caa cag gag cgg cag ttc gag gtc aag ttt gaa gtc tcc aaa      2592
Ala Cys Gln Gln Glu Arg Gln Phe Glu Val Lys Phe Glu Val Ser Lys
    850                 855                 860
```

```
tgg gag aat ccc cgc gtg ctg agc ttc tca ctg aca tcc cag acg atg      2640
Trp Glu Asn Pro Arg Val Leu Ser Phe Ser Leu Thr Ser Gln Thr Met
865                 870                 875                 880

ctg gac cag agt gtg gac ttt gat gtg ctg cca gcc ttt gac gcc cta      2688
Leu Asp Gln Ser Val Asp Phe Asp Val Leu Pro Ala Phe Asp Ala Leu
                885                 890                 895

ggc cag ctg gtc tct ggc tcc agg ccc agc tct caa gtc tac gtc gac      2736
Gly Gln Leu Val Ser Gly Ser Arg Pro Ser Ser Gln Val Tyr Val Asp
            900                 905                 910

ctc atc cac agc tac agc aat gcg ggc gag tac tcc acc tgc ttc aca      2784
Leu Ile His Ser Tyr Ser Asn Ala Gly Glu Tyr Ser Thr Cys Phe Thr
        915                 920                 925

gag cta caa cgg gac ttc atc atc tct cgc cct acc aag ctg aag agc      2832
Glu Leu Gln Arg Asp Phe Ile Ile Ser Arg Pro Thr Lys Leu Lys Ser
    930                 935                 940

ctg atc cgg ctg gtg aag cac tgg tac cag cag tgt acc aag atc tcc      2880
Leu Ile Arg Leu Val Lys His Trp Tyr Gln Gln Cys Thr Lys Ile Ser
945                 950                 955                 960

aag ggg aga ggc tcc cta ccc cca cag cac ggg ctg gaa ctc ctg act      2928
Lys Gly Arg Gly Ser Leu Pro Pro Gln His Gly Leu Glu Leu Leu Thr
                965                 970                 975

gtg tat gcc tgg gag cag ggc ggg aag gac tcc cag ttc aac atg gct      2976
Val Tyr Ala Trp Glu Gln Gly Gly Lys Asp Ser Gln Phe Asn Met Ala
            980                 985                 990

gag ggc ttc cgc acg gtc ctg gag  ctg gtc acc cag tac  cgc cag ctc    3024
Glu Gly Phe Arg Thr Val Leu Glu  Leu Val Thr Gln Tyr  Arg Gln Leu
        995                 1000                 1005

tgt atc  tac tgg acc atc aac  tac aac gcc aag gac  aag act gtt       3069
Cys Ile  Tyr Trp Thr Ile Asn  Tyr Asn Ala Lys Asp  Lys Thr Val
    1010                 1015                 1020

gga gac  ttc ctg aaa cag cag  ctt cag aag ccc agg  cct atc atc       3114
Gly Asp  Phe Leu Lys Gln Gln  Leu Gln Lys Pro Arg  Pro Ile Ile
    1025                 1030                 1035

ctg gat  ccg gct gac ccg aca  ggc aac ctg ggc cac  aat gcc cgc       3159
Leu Asp  Pro Ala Asp Pro Thr  Gly Asn Leu Gly His  Asn Ala Arg
    1040                 1045                 1050

tgg gac  ctg ctg gcc aag gaa  gct gca gcc tgc aca  tct gcc ctg       3204
Trp Asp  Leu Leu Ala Lys Glu  Ala Ala Ala Cys Thr  Ser Ala Leu
    1055                 1060                 1065

tgc tgc  atg gga cgg aat ggc  atc ccc atc cag cca  tgg cca gtg       3249
Cys Cys  Met Gly Arg Asn Gly  Ile Pro Ile Gln Pro  Trp Pro Val
    1070                 1075                 1080

aag gct  gct gtg tga                                                3264
Lys Ala  Ala Val
    1085
```

<210> 10
<211> 1087
<212> PRT
<213> Homo sapiens

<400> 10

```
Met Asp Leu Tyr Ser Thr Pro Ala Ala Ala Leu Asp Arg Phe Val Ala
1               5                   10                  15

Arg Arg Leu Gln Pro Arg Lys Glu Phe Val Glu Lys Ala Arg Arg Ala
            20                  25                  30

Leu Gly Ala Leu Ala Ala Ala Leu Arg Glu Arg Gly Gly Arg Leu Gly
        35                  40                  45

Ala Ala Ala Pro Arg Val Leu Lys Thr Val Lys Gly Gly Ser Ser Gly
    50                  55                  60

Arg Gly Thr Ala Leu Lys Gly Gly Cys Asp Ser Glu Leu Val Ile Phe
65                  70                  75                  80

Leu Asp Cys Phe Lys Ser Tyr Val Asp Gln Arg Ala Arg Arg Ala Glu
                85                  90                  95

Ile Leu Ser Glu Met Arg Ala Ser Leu Glu Ser Trp Trp Gln Asn Pro
            100                 105                 110

Val Pro Gly Leu Arg Leu Thr Phe Pro Glu Gln Ser Val Pro Gly Ala
        115                 120                 125

Leu Gln Phe Arg Leu Thr Ser Val Asp Leu Glu Asp Trp Met Asp Val
    130                 135                 140

Ser Leu Val Pro Ala Phe Asn Val Leu Gly Gln Ala Gly Ser Ala Val
145                 150                 155                 160

Lys Pro Lys Pro Gln Val Tyr Ser Thr Leu Leu Asn Ser Gly Cys Gln
                165                 170                 175

Gly Gly Glu His Ala Ala Cys Phe Thr Glu Leu Arg Arg Asn Phe Val
            180                 185                 190

Asn Ile Arg Pro Ala Lys Leu Lys Asn Leu Ile Leu Leu Val Lys His
        195                 200                 205

Trp Tyr His Gln Val Cys Leu Gln Gly Leu Trp Lys Glu Thr Leu Pro
    210                 215                 220
```

Pro Val Tyr Ala Leu Glu Leu Leu Thr Ile Phe Ala Trp Glu Gln Gly
225 230 235 240

Cys Lys Lys Asp Ala Phe Ser Leu Gly Glu Gly Leu Arg Thr Val Leu
245 250 255

Gly Leu Ile Gln Gln His Gln His Leu Cys Val Phe Trp Thr Val Asn
260 265 270

Tyr Gly Phe Glu Asp Pro Ala Val Gly Gln Phe Leu Gln Arg His Val
275 280 285

Lys Arg Pro Arg Pro Val Ile Leu Asp Pro Ala Asp Pro Thr Trp Asp
290 295 300

Leu Gly Asn Gly Ala Ala Trp His Trp Asp Leu His Ala Gln Glu Ala
305 310 315 320

Ala Ser Cys Tyr Asp His Pro Cys Phe Leu Arg Gly Met Gly Asp Pro
325 330 335

Val Gln Ser Trp Lys Gly Pro Gly Leu Pro Arg Ala Gly Cys Ser Gly
340 345 350

Leu Gly His Pro Ile Gln Leu Asp Pro Asn Gln Lys Thr Pro Glu Asn
355 360 365

Ser Lys Ser Leu Asn Ala Val Tyr Pro Arg Ala Gly Ser Lys Pro Pro
370 375 380

Ser Cys Pro Ala Pro Gly Pro Thr Ala Glu Pro Ala Ser Tyr Pro Ser
385 390 395 400

Val Pro Gly Met Ala Leu Asp Leu Ser Gln Ile Pro Thr Lys Glu Leu
405 410 415

Asp Arg Phe Ile Gln Asp His Leu Lys Pro Ser Pro Gln Phe Gln Glu
420 425 430

Gln Val Lys Lys Ala Ile Asp Ile Ile Leu Arg Cys Leu His Glu Asn
435 440 445

Cys Val His Lys Ala Ser Arg Val Ser Lys Gly Gly Ser Phe Gly Arg
450 455 460

```
Gly Thr Asp Leu Arg Asp Gly Cys Asp Val Glu Leu Ile Ile Phe Leu
465                 470                 475                 480

Asn Cys Phe Thr Asp Tyr Lys Asp Gln Gly Pro Arg Arg Ala Glu Ile
                485                 490                 495

Leu Asp Glu Met Arg Ala His Val Glu Ser Trp Trp Gln Asp Gln Val
            500                 505                 510

Pro Ser Leu Ser Leu Gln Phe Pro Glu Gln Asn Val Pro Glu Ala Leu
        515                 520                 525

Gln Phe Gln Leu Val Ser Thr Ala Leu Lys Ser Trp Thr Asp Val Ser
    530                 535                 540

Leu Leu Pro Ala Phe Asp Ala Val Gly Gln Leu Ser Ser Gly Thr Lys
545                 550                 555                 560

Pro Asn Pro Gln Val Tyr Ser Arg Leu Leu Thr Ser Gly Cys Gln Glu
                565                 570                 575

Gly Glu His Lys Ala Cys Phe Ala Glu Leu Arg Arg Asn Phe Met Asn
            580                 585                 590

Ile Arg Pro Val Lys Leu Lys Asn Leu Ile Leu Leu Val Lys His Trp
        595                 600                 605

Tyr Arg Gln Val Ala Ala Gln Asn Lys Gly Lys Gly Pro Ala Pro Ala
    610                 615                 620

Ser Leu Pro Pro Ala Tyr Ala Leu Glu Leu Leu Thr Ile Phe Ala Trp
625                 630                 635                 640

Glu Gln Gly Cys Arg Gln Asp Cys Phe Asn Met Ala Gln Gly Phe Arg
                645                 650                 655

Thr Val Leu Gly Leu Val Gln Gln His Gln Gln Leu Cys Val Tyr Trp
            660                 665                 670

Thr Val Asn Tyr Ser Thr Glu Asp Pro Ala Met Arg Met His Leu Leu
        675                 680                 685

Gly Gln Leu Arg Lys Pro Arg Pro Leu Val Leu Asp Pro Ala Asp Pro
    690                 695                 700
```

Thr Trp Asn Val Gly His Gly Ser Trp Glu Leu Leu Ala Gln Glu Ala
705 710 715 720

Ala Ala Leu Gly Met Gln Ala Cys Phe Leu Ser Arg Asp Gly Thr Ser
725 730 735

Val Gln Pro Trp Asp Val Met Pro Ala Leu Leu Tyr Gln Thr Pro Ala
740 745 750

Gly Asp Leu Asp Lys Phe Ile Ser Glu Phe Leu Gln Pro Asn Arg Gln
755 760 765

Phe Leu Ala Gln Val Asn Lys Ala Val Asp Thr Ile Cys Ser Phe Leu
770 775 780

Lys Glu Asn Cys Phe Arg Asn Ser Pro Ile Lys Val Ile Lys Val Val
785 790 795 800

Lys Gly Gly Ser Ser Ala Lys Gly Thr Ala Leu Arg Gly Arg Ser Asp
805 810 815

Ala Asp Leu Val Val Phe Leu Ser Cys Phe Ser Gln Phe Thr Glu Gln
820 825 830

Gly Asn Lys Arg Ala Glu Ile Ile Ser Glu Ile Arg Ala Gln Leu Glu
835 840 845

Ala Cys Gln Gln Glu Arg Gln Phe Glu Val Lys Phe Glu Val Ser Lys
850 855 860

Trp Glu Asn Pro Arg Val Leu Ser Phe Ser Leu Thr Ser Gln Thr Met
865 870 875 880

Leu Asp Gln Ser Val Asp Phe Asp Val Leu Pro Ala Phe Asp Ala Leu
885 890 895

Gly Gln Leu Val Ser Gly Ser Arg Pro Ser Ser Gln Val Tyr Val Asp
900 905 910

Leu Ile His Ser Tyr Ser Asn Ala Gly Glu Tyr Ser Thr Cys Phe Thr
915 920 925

Glu Leu Gln Arg Asp Phe Ile Ile Ser Arg Pro Thr Lys Leu Lys Ser
930 935 940

Leu Ile Arg Leu Val Lys His Trp Tyr Gln Gln Cys Thr Lys Ile Ser

```
945                 950                 955                 960

Lys Gly Arg Gly Ser Leu Pro Pro Gln His Gly Leu Glu Leu Leu Thr
                965                 970                 975

Val Tyr Ala Trp Glu Gln Gly Gly Lys Asp Ser Gln Phe Asn Met Ala
            980                 985                 990

Glu Gly Phe Arg Thr Val Leu Glu  Leu Val Thr Gln Tyr  Arg Gln Leu
        995                 1000                1005

Cys Ile  Tyr Trp Thr Ile Asn  Tyr Asn Ala Lys Asp  Lys Thr Val
    1010                 1015                 1020

Gly Asp  Phe Leu Lys Gln Gln  Leu Gln Lys Pro Arg  Pro Ile Ile
    1025                 1030                 1035

Leu Asp  Pro Ala Asp Pro Thr  Gly Asn Leu Gly His  Asn Ala Arg
    1040                 1045                 1050

Trp Asp  Leu Leu Ala Lys Glu  Ala Ala Ala Cys Thr  Ser Ala Leu
    1055                 1060                 1065

Cys Cys  Met Gly Arg Asn Gly  Ile Pro Ile Gln Pro  Trp Pro Val
    1070                 1075                 1080

Lys Ala  Ala Val
    1085
```

<210> 11
<211> 1104
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(1104)
<223>

<300>
<301> Coccia, E.M., et al.
<302> A full-length murine 2-5A synthetase cDNA transfected in NIH-3T3 cells
<303> Virology
<304> 179
<305> 1
<306> 228-233
<307> 1990
<308> NCBI/M33863
<309> 1993-06-11

```
<400>  11
atg gag cac gga ctc agg agc atc cca gcc tgg acg ctg gac aag ttc        48
Met Glu His Gly Leu Arg Ser Ile Pro Ala Trp Thr Leu Asp Lys Phe
1               5                   10                  15

ata gag gat tac ctc ctt ccc gac acc acc ttt ggt gct gat gtc aaa        96
Ile Glu Asp Tyr Leu Leu Pro Asp Thr Thr Phe Gly Ala Asp Val Lys
            20                  25                  30

tca gcc gtc aat gtc gtg tgt gat ttc ctg aag gag aga tgc ttc caa       144
Ser Ala Val Asn Val Val Cys Asp Phe Leu Lys Glu Arg Cys Phe Gln
        35                  40                  45

ggt gct gcc cac cca gtg agg gtc tcc aag gtg gtg aag ggt ggc tcc       192
Gly Ala Ala His Pro Val Arg Val Ser Lys Val Val Lys Gly Gly Ser
    50                  55                  60

tca ggc aaa ggc acc aca ctc aag ggc agg tca gac gct gac ctg gtg       240
Ser Gly Lys Gly Thr Thr Leu Lys Gly Arg Ser Asp Ala Asp Leu Val
65                  70                  75                  80

gtg ttc ctt aac aat ctc acc agc ttt gag gat cag tta aac cga cgg       288
Val Phe Leu Asn Asn Leu Thr Ser Phe Glu Asp Gln Leu Asn Arg Arg
                85                  90                  95

gga gag ttc atc aag gaa att aag aaa cag ctg tac gag gtt cag cat       336
Gly Glu Phe Ile Lys Glu Ile Lys Lys Gln Leu Tyr Glu Val Gln His
            100                 105                 110

gag aga cgt ttt aga gtc aag ttt gag gtc cag agt tca tgg tgg ccc       384
Glu Arg Arg Phe Arg Val Lys Phe Glu Val Gln Ser Ser Trp Trp Pro
        115                 120                 125

aac gcc cgg tct ctg agc ttc aag ctg agc gcc ccc cat ctg cat cag       432
Asn Ala Arg Ser Leu Ser Phe Lys Leu Ser Ala Pro His Leu His Gln
    130                 135                 140

gag gtg gag ttt gat gtg ctg cca gcc ttt gat gtc ctg ggt cat gtt       480
Glu Val Glu Phe Asp Val Leu Pro Ala Phe Asp Val Leu Gly His Val
145                 150                 155                 160

aat act tcc agc aag cct gat ccc aga atc tat gcc atc ctc atc gag       528
Asn Thr Ser Ser Lys Pro Asp Pro Arg Ile Tyr Ala Ile Leu Ile Glu
                165                 170                 175

gaa tgt acc tcc ctg ggg aag gat ggc gag ttc tct acc tgc ttc acg       576
Glu Cys Thr Ser Leu Gly Lys Asp Gly Glu Phe Ser Thr Cys Phe Thr
            180                 185                 190

gag ctc cag cgg aac ttc ctg aag cag cgc cca acc aag ctg aag agt       624
Glu Leu Gln Arg Asn Phe Leu Lys Gln Arg Pro Thr Lys Leu Lys Ser
        195                 200                 205

ctc atc cgc ctg gtc aag cac tgg tac caa ctg tgt aag gag aag ctg       672
Leu Ile Arg Leu Val Lys His Trp Tyr Gln Leu Cys Lys Glu Lys Leu
    210                 215                 220

ggg aag cca ttg cct cca cag tac gcc cta gag ttg ctc act gtc ttt       720
Gly Lys Pro Leu Pro Pro Gln Tyr Ala Leu Glu Leu Leu Thr Val Phe
225                 230                 235                 240
```

```
gcc tgg gaa caa ggg aat gga tgt tat gag ttc aac aca gcc cag ggc      768
Ala Trp Glu Gln Gly Asn Gly Cys Tyr Glu Phe Asn Thr Ala Gln Gly
                245                 250                 255

ttc cgg acc gtc ttg gaa ctg gtc atc aat tat cag cat ctt cga atc      816
Phe Arg Thr Val Leu Glu Leu Val Ile Asn Tyr Gln His Leu Arg Ile
            260                 265                 270

tac tgg aca aag tat tat gac ttt caa cac cag gag gtc tcc aaa tac      864
Tyr Trp Thr Lys Tyr Tyr Asp Phe Gln His Gln Glu Val Ser Lys Tyr
        275                 280                 285

ctg cac aga cag ctc aga aaa gcc agg cct gtg atc ctg gac cca gct      912
Leu His Arg Gln Leu Arg Lys Ala Arg Pro Val Ile Leu Asp Pro Ala
    290                 295                 300

gac cca aca ggg aat gtg gcc ggt ggg aac cca gag ggc tgg agg cgg      960
Asp Pro Thr Gly Asn Val Ala Gly Gly Asn Pro Glu Gly Trp Arg Arg
305                 310                 315                 320

ttg gct gaa gag gct gat gtg tgg cta tgg tac cca tgt ttt att aaa     1008
Leu Ala Glu Glu Ala Asp Val Trp Leu Trp Tyr Pro Cys Phe Ile Lys
                325                 330                 335

aag gat ggt tcc cga gtg agc tcc tgg gat gtg ccg acg gtg gtt cct     1056
Lys Asp Gly Ser Arg Val Ser Ser Trp Asp Val Pro Thr Val Val Pro
            340                 345                 350

gta cct ttt gag cag gta gaa gag aac tgg aca tgt atc ctg ctg tga     1104
Val Pro Phe Glu Gln Val Glu Glu Asn Trp Thr Cys Ile Leu Leu
        355                 360                 365


<210>  12
<211>  367
<212>  PRT
<213>  Mus musculus

<400>  12

Met Glu His Gly Leu Arg Ser Ile Pro Ala Trp Thr Leu Asp Lys Phe
1               5                   10                  15

Ile Glu Asp Tyr Leu Leu Pro Asp Thr Thr Phe Gly Ala Asp Val Lys
            20                  25                  30

Ser Ala Val Asn Val Val Cys Asp Phe Leu Lys Glu Arg Cys Phe Gln
        35                  40                  45

Gly Ala Ala His Pro Val Arg Val Ser Lys Val Val Lys Gly Gly Ser
    50                  55                  60

Ser Gly Lys Gly Thr Thr Leu Lys Gly Arg Ser Asp Ala Asp Leu Val
65                  70                  75                  80

Val Phe Leu Asn Asn Leu Thr Ser Phe Glu Asp Gln Leu Asn Arg Arg
```

85 90 95

Gly Glu Phe Ile Lys Glu Ile Lys Lys Gln Leu Tyr Glu Val Gln His
100 105 110

Glu Arg Arg Phe Arg Val Lys Phe Glu Val Gln Ser Ser Trp Trp Pro
115 120 125

Asn Ala Arg Ser Leu Ser Phe Lys Leu Ser Ala Pro His Leu His Gln
130 135 140

Glu Val Glu Phe Asp Val Leu Pro Ala Phe Asp Val Leu Gly His Val
145 150 155 160

Asn Thr Ser Ser Lys Pro Asp Pro Arg Ile Tyr Ala Ile Leu Ile Glu
165 170 175

Glu Cys Thr Ser Leu Gly Lys Asp Gly Glu Phe Ser Thr Cys Phe Thr
180 185 190

Glu Leu Gln Arg Asn Phe Leu Lys Gln Arg Pro Thr Lys Leu Lys Ser
195 200 205

Leu Ile Arg Leu Val Lys His Trp Tyr Gln Leu Cys Lys Glu Lys Leu
210 215 220

Gly Lys Pro Leu Pro Pro Gln Tyr Ala Leu Glu Leu Leu Thr Val Phe
225 230 235 240

Ala Trp Glu Gln Gly Asn Gly Cys Tyr Glu Phe Asn Thr Ala Gln Gly
245 250 255

Phe Arg Thr Val Leu Glu Leu Val Ile Asn Tyr Gln His Leu Arg Ile
260 265 270

Tyr Trp Thr Lys Tyr Tyr Asp Phe Gln His Gln Glu Val Ser Lys Tyr
275 280 285

Leu His Arg Gln Leu Arg Lys Ala Arg Pro Val Ile Leu Asp Pro Ala
290 295 300

Asp Pro Thr Gly Asn Val Ala Gly Gly Asn Pro Glu Gly Trp Arg Arg
305 310 315 320

Leu Ala Glu Glu Ala Asp Val Trp Leu Trp Tyr Pro Cys Phe Ile Lys
325 330 335

Lys Asp Gly Ser Arg Val Ser Ser Trp Asp Val Pro Thr Val Val Pro
            340   345   350

Val Pro Phe Glu Gln Val Glu Glu Asn Trp Thr Cys Ile Leu Leu
        355   360   365

<210> 13
<211> 1590
<212> DNA
<213> Homo sapiens

<300>
<301> Aissouni, Y. et al.
<302> The cleavage/polyadenylation activity triggered by a U-rich motif sequence
<303> J. Biol. Chem.
<304> 277
<305> 39
<306> 35808-35814
<307> 2002
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Behera, A.K., et al.
<302> 2'-5' Oligoadenylate synthetase plays a critical role in interferon-gamma inhibition
<303> J. Biol. Chem.
<304> 277
<305> 28
<306> 25601-25608
<307> 2002
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Sarkar, S.N., et al.
<302> Identification of the substrate-binding sites of 2'-5'-oligoadenylate synthetase
<303> J. Biol. Chem.
<304> 277
<305> 27
<306> 24321-24330
<307> 2002
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Hovnanian, A., et al.
<302> The human 2',5'-oligoadenylate synthetase locus is composed of three distinct genes
<303> Genomics
<304> 52
<305> 3
<306> 267-277
<307> 1998
<308> NCBI/NM_016816

<309> 2003-04-06

<300>
<301> Renault, B.
<302> A sequence-ready physical map of a region of 12q24.1
<303> Genomics
<304> 45
<305> 2
<306> 271-278
<307> 1997
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Nechiporuk, T., et al.
<302> A high-resolution PAC and BAC map of the SCA2 region
<303> Genomics
<304> 44
<305> 3
<306> 321-329
<307> 1997
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Wathelet, M.G., et al.
<302> Cloning and chromosomal location of human genes inducible by type I interferon
<303> Somat. Cell Mol. Genet.
<304> 14
<305> 5
<306> 415-426
<307> 1988
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Rutherford, M.N., et al.
<302> Interferon-induced binding of nuclear factors to promoter elements of the 2-5A synthetase gene
<303> EMBO J.
<304> 7
<305> 3
<306> 751-759
<307> 1988
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Wathelet, M.G., et al.
<302> New inducers revealed by the promoter sequence analysis of two interferon-activated human genes
<303> Eur. J. Biochem.
<304> 169
<305> 2
<306> 313-321
<307> 1987
<308> NCBI/NM_016816
<309> 2003-04-06

<300>

<301> Benech, P., et al.
<302> Interferon-responsive regulatory elements in the promoter of the human 2',5'-oligo(A) synthetase gene
<303> Mol. Cell. Biol.
<304> 7
<305> 12
<306> 4498-4504
<307> 1987
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Hovanessian, A.G., et al.
<302> Identification of 69-kd and 100-kd forms of 2-5A sythetase
<303> EMBO J.
<304> 6
<305> 5
<306> 1273-1280
<307> 1987
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Williams, B.R., et al.
<302> Interferon-regulated human 2-5A synthetase gene maps to chromosome
<303> Somat. Cell Mol. Genet.
<304> 12
<305> 4
<306> 403-408
<307> 1986
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Shiojiri, S., et al.
<302> Structure and expression of a cloned cDNA
<303> J. Biochem.
<304> 99
<305> 5
<306> 1455-1464
<307> 1986
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Wathelet, M., et al.
<302> Full-length sequence and expression of the 42 kDa 2-5A synthetase
<303> FEBS Lett.
<304> 196
<305> 1
<306> 113-120
<307> 1986
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Benech, P., et al.
<302> Structure of two forms of the interferon-induced (2'-5') oligo A synthetase
<303> EMBO J.
<304> 4

<305> 9
<306> 2249-2256
<307> 1985
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Saunders, M.E., et al.
<302> Human 2-5A synthetase: characterization of a novel cDNA and corresponding gene structure
<303> EMBO J.
<304> 4
<305> 7
<306> 1761-1768
<307> 1985
<308> NCBI/NM_016816
<309> 2003-04-06

<300>
<301> Merlin, G., et al.
<302> Molecular cloning and sequence of partial cDNA for interferon-induced (2'-5') oligo(A) synthetase mRNA from human cells
<303> Proc. Natl. Acad. Sci. U.S.A.
<304> 80
<305> 16
<306> 4904-4908
<307> 1983
<308> NCBI/NM_016816
<309> 2003-04-06

<400> 13
gaggcagttc tgttgccact ctctctcctg tcaatgatgg atctcagaaa taccccagcc 60

aaatctctgg acaagttcat tgaagactat ctcttgccag acacgtgttt ccgcatgcaa 120

atcgaccatg ccattgacat catctgtggg ttcctgaagg aaaggtgctt ccgaggtagc 180

tcctaccctg tgtgtgtgtc caaggtggta aagggtggct cctcaggcaa gggcaccacc 240

ctcagaggcc gatctgacgc tgacctggtt gtcttcctca gtcctctcac cacttttcag 300

gatcagttaa atcgccgggg agagttcatc caggaaatta ggagacagct ggaagcctgt 360

caaagagaga gagcactttc cgtgaagttt gaggtccagg ctccacgctg gggcaacccc 420

cgtgcgctca gcttcgtact gagttcgctc cagctcgggg agggggtgga gttcgatgtg 480

ctgcctgcct ttgatgccct gggtcagttg actggcagct ataaacctaa cccccaaatc 540

tatgtcaagc tcatcgagga gtgcaccgac ctgcagaaag agggcgagtt ctccacctgc 600

ttcacagaac tacagagaga cttcctgaag cagcgcccca ccaagctcaa gagcctcatc 660

cgcctagtca agcactggta ccaaaattgt aagaagaagc ttgggaagct gccacctcag 720

tatgccctgg agctcctgac ggtctatgct tgggagcgag ggagcatgaa aacacatttc 780

aacacagccc aaggatttcg gacggtcttg gaattagtca taaactacca gcaactctgc 840

atctactgga caaagtatta tgactttaaa aaccccatta ttgaaaagta cctgagaagg 900

cagctcacga aacc[illegible]gcc tgtgatcctg gacccggcgg accctacagg a[illegible]cttgggt 960
ggtggagacc caaagggttg gaggcagctg gcacaagagg ctgaggcctg gctgaattac 1020
ccatgcttta agaattggga tgggtcccca gtgagctcct ggattctgct ggctgaaagc 1080
aacagtacag acgatgagac cgacgatccc aggacgtatc agaaatatgg ttacattgga 1140
acacatgagt accctcattt ctctcataga cccagcacgc tccaggcagc atccacccca 1200
caggcagaag aggactggac ctgcaccatc ctctgaatgc cagtgcatct tgggggaaag 1260
ggctccagtg ttatctggac cagttccttc attttcaggt gggactcttg atccagagaa 1320
gacaaagctc ctcagtgagc tggtgtataa tccaagacag aacccaagtc tcctgactcc 1380
tggccttcta tgccctctat cctatcatag ataacattct ccacagcctc acttcattcc 1440
acctattctc tgaaaatatt ccctgagaga gaacagagag atttagataa gagaatgaaa 1500
ttccagcctt gactttcttc tgtgcacctg atgggagggt aatgtctaat gtattatcaa 1560
taacaataaa aataaagcaa ataccaaaaa 1590

<210> 14
<211> 3068
<212> DNA
<213> Homo sapiens

<300>
<301> Hovnanian, A., et al.
<302> The human 2',5'-oligoadenylate synthetase locus is composed of three distinct genes
<303> Genomics
<304> 52
<305> 3
<306> 267-277
<307> 1998
<308> NCBI/NM_002535
<309> 2003-04-03

<300>
<301> Marie, I. and Hovanessian, A.G.
<302> The 69-kDa 2-5A synthetase is composed of two homologous and adjacent functional domains
<303> J. Biol. Chem.
<304> 267
<305> 14
<306> 9933-9939
<307> 1992
<308> NCBI/NM_002535
<309> 2003-04-03

<300>
<301> Marie, I., et al.
<302> Differential expression and distinct structure of 69- and 100-kDa forms of 2-5A synthetase
<303> J. Biol. Chem.
<304> 265
<305> 30

<306> 18601-18607
<307> 1990
<308> NCBI/NM_002535
<309> 2003-04-03

<300>
<301> Marie, I., et al.
<302> Preparation and characterization of polyclonal antibodies
<303> Biochem. Biophys. Res. Commun.
<304> 160
<305> 2
<306> 580-587
<307> 1989
<308> NCBI/NM_002535
<309> 2003-04-03

<300>
<301> Hovanessian, A.G., et al.
<302> Characterization of 69- and 100-kDa forms of 2-5A-synthetase
<303> J. Biol. Chem.
<304> 263
<305> 10
<306> 4959
<307> 1988
<308> NCBI/NM_002535
<309> 2003-04-03

<300>
<301> Hovanessian, A.G., et al.
<302> Identification of 69-kd and 100-kd forms of 2-5A synthetase
<303> EMBO J.
<304> 6
<305> 5
<306> 1273-1280
<307> 1987
<308> NCBI/NM_002535
<309> 2003-04-03

<400> 14
cggcagccag ctgagagcaa tgggaaatgg ggagtcccag ctgtcctcgg tgcctgctca 60

gaagctgggt tggtttatcc aggaatacct gaagccctac gaagaatgtc agacactgat 120

cgacgagatg gtgaacacca tctgtgacgt ctgcaggaac cccgaacagt tccccctggt 180

gcagggagtg gccataggtg gctcctatgg acggaaaaca gtcttaagag gcaactccga 240

tggtaccctt gtcctttttct tcagtgactt aaaacaattc caggatcaga agagaagcca 300

acgtgacatc ctcgataaaa ctggggataa gctgaagttc tgtctgttca cgaagtggtt 360

gaaaaacaat ttcgagatcc agaagtccct tgatgggtcc accatccagg tgttcacaaa 420

aaatcagaga atctctttcg aggtgctggc cgccttcaac gctctgagct taaatgataa 480

tcccagcccc tggatctatc gagagctcaa aagatccttg gataagacaa atgccagtcc 540

tggtgagttt gcagtctgct tcactgaact ccagcagaag ttttttgaca accgtcctgg 600

aaaactaaag gatttgatcc tcttgataaa gcactggcat caacagtgcc agaaaaaaat 660

caaggattta ccctcgctgt ctccgtatgc cctggagctg cttacggtgt atgcctggga 720
acaggggtgc agaaaagaca actttgacat tgctgaaggc gtcagaacgg ttctggagct 780
gatcaaatgc caggagaagc tgtgtatcta ttggatggtc aactacaact ttgaagatga 840
gaccatcagg aacatcctgc tgcaccagct ccaatcagcg aggccagtaa tcttggatcc 900
agttgaccca accaataatg tgagtggaga taaaatatgc tggcaatggc tgaaaaaaga 960
agctcaaacc tggttgactt ctcccaacct ggataatgag ttacctgcac catcttggaa 1020
tgtcctgcct gcaccactct tcacgacccc aggccacctt ctggataagt tcatcaagga 1080
gtttctccag cccaacaaat gcttcctaga gcagattgac agtgctgtta acatcatccg 1140
tacattcctt aaagaaaact gcttccgaca atcaacagcc aagatccaga ttgtccgggg 1200
aggatcaacc gccaaaggca cagctctgaa gactggctct gatgccgatc tcgtcgtgtt 1260
ccataactca cttaaaagct acacctccca aaaaaacgag cggcacaaaa tcgtcaagga 1320
aatccatgaa cagctgaaag ccttttggag ggagaaggag gaggagcttg aagtcagctt 1380
tgagcctccc aagtggaagg ctcccagggt gctgagcttc tctctgaaat ccaaagtcct 1440
caacgaaagt gtcagctttg atgtgcttcc tgcctttaat gcactgggtc agctgagttc 1500
tggctccaca cccagccccg aggtttatgc agggctcatt gatctgtata aatcctcgga 1560
cctcccggga ggagagtttt ctacctgttt cacagtcctg cagcgaaact tcattcgctc 1620
ccggcccacc aaactaaagg atttaattcg cctggtgaag cactggtaca aagagtgtga 1680
aaggaaactg aagccaaagg ggtctttgcc cccaaagtat gccttggagc tgctcaccat 1740
ctatgcctgg gagcagggga gtggagtgcc ggattttgac actgcagaag gtttccggac 1800
agtcctggag ctggtcacac aatatcagca gctcggcatc ttctggaagg tcaattacaa 1860
ctttgaagat gagaccgtga ggaagtttct actgagccag ttgcagaaaa ccaggcctgt 1920
gatcttggac ccaggcgaac ccacaggtga cgtgggtgga ggggaccgtt ggtgttggca 1980
tcttctggac aaagaagcaa aggttaggtt atcctctccc tgcttcaagg atgggactgg 2040
aaacccaata ccaccttgga aagtgccggt aaaagtcatc taaaggaggc gttgtctgga 2100
aatagccctg taacaggctt gaatcaaaga acttctccta ctgtagcaac ctgaaattaa 2160
ctcagacaca aataaaggaa acccagctca caggagctta aacagctggt cagcccccct 2220
aagcccccac tacaagtgat cctcaggcag gtaaccccag attcatgcac tgtagggctg 2280
ggcgcagcat ccctaggtct ctacccagta gatgccacta gccctcctct cccagtgaca 2340
accaaaagtc ttcacatgtt caaacgttcc cctgggttca cagatctttc tgcctttggc 2400
ttttggctcc accctcttta gctgttaatt tgagtactta tggccctgaa agcggccacg 2460
gtgcctccag atggcaggtt tgcaatccaa gcaggaagaa ggaaaagata cccaaaggtc 2520

aagaacacag tgattttatt agaagtttca tccgcaaatt ttcttccatt tcattgctca 2580

gaatgtcatg tggttacctg taacttgaag gtggctacaa agatgactgt ggaggtggtt 2640

gcacttgcca cccaaggatg tctgccacac ctctccaagc cctcctacct accaagatat 2700

acctgatata tccaccagat atctcctcag atatacttgg ttctctccac caggttcttt 2760

ctttaaagca ggattctcaa ctttgatact tactcacatt gggctagaca gttctttgtt 2820

tggaggctct cttgtgcatg taggatgttg agcagcatgt gtggcctgta cccagtacat 2880

gccacccagt tgtgacaatt aaaagtgtct tgagacttta tcatgtgtct tctgccctag 2940

gtgagaaccc ttgcactaca ggaaccctac acccaacctg gggggaatgt agggaagagg 3000

tgccaagcca accgtggggt tagctctaat tattaagtta tgcattataa ataaatacca 3060

aaaaattg 3068

<210> 15
<211> 6270
<212> DNA
<213> Homo sapiens

<300>
<301> Rebouillat, D., et al.
<302> The 100-kDa 2',5'-oligoadenylate synthetase catalyzing preferentially the synthesis of dimeric pppA2'p5'A molecules
<303> J. Biol. Chem.
<304> 274
<305> 3
<306> 1557-1565
<307> 1999
<308> NCBI/AF063613
<309> 1999-05-04

<300>
<301> Rebouillat, D., and Hovanessian, A.G.
<302> Direct Submission
<303> Submitted (07-May-1998) Dept. of AIDS and Retroviruses, Institut Pasteur
<304> 0
<305> 0
<306> 0
<307> 1998
<308> NCBI/AF063613
<309> 1999-05-04

<400> 15
gccctgcttc cccttgcacc tgcgccgggc ggccatggac ttgtacagca ccccggccgc 60

tgcgctggac aggttcgtgg ccagaaggct gcagccgcgg aaggagttcg tagagaaggc 120

gcggcgcgct ctgggcgccc tggccgctgc cctgagggag cgcgggggcc gcctcggtgc 180

tgctgccccg cgggtgctga aaactgtcaa gggaggctcc tcgggccggg gcacagctct 240

caagggtggc tgtgattctg aacttgtcat cttcctcgac tgcttcaaga gctatgtgga 300

ccagagggcc cgccgtgcag agatcctcag tgagatgcgg gcatcgctgg aatcctggtg 360
gcagaaccca gtccctggtc tgagactcac gtttcctgag cagagcgtgc ctggggccct 420
gcagttccgc ctgacatccg tagatcttga ggactggatg gatgttagcc tggtgcctgc 480
cttcaatgtc ctgggtcagg ccggctccgc ggtcaaaccc aagccacaag tctactctac 540
cctcctcaac agtggctgcc aagggggcga gcatgcggcc tgcttcacag agctgcggag 600
gaactttgtg aacattcgcc cagccaagtt gaagaaccta atcttgctgg tgaagcactg 660
gtaccaccag gtgtgcctac aggggttgtg gaaggagacg ctgcccccgg tctatgccct 720
ggaattgctg accatcttcg cctgggagca gggctgtaag aaggatgctt tcagcctagg 780
cgaaggcctc cgaactgtcc tgggcctgat ccaacagcat cagcacctgt gtgttttctg 840
gactgtcaac tatggcttcg aggaccctgc agttgggcag ttcttgcagc ggcacgttaa 900
gagacccagg cctgtgatcc tggacccagc tgaccccaca tgggacctgg ggaatggggc 960
agcctggcac tgggatttgc atgcccagga ggcagcatcc tgctatgacc acccatgctt 1020
tctgaggggg atgggggacc cagtgcagtc ttggaagggg ccgggccttc cacgtgctgg 1080
atgctcaggt ttgggccacc ccatccagct agaccctaac cagaagaccc ctgaaaacag 1140
caagagcctc aatgctgtgt acccaagagc agggagcaaa cctccctcat gcccagctcc 1200
tggccccact gcggagccag catcgtaccc ctctgtgccg ggaatggcct tggacctgtc 1260
tcagatcccc accaaggagc tggaccgctt catccaggac cacctgaagc cgagccccca 1320
gttccaggag caggtgaaaa aggccatcga catcatcttg cgctgcctcc atgagaactg 1380
tgttcacaag gcctcaagag tcagtaaagg gggctcattt ggccggggca cagacctaag 1440
ggatggctgt gatgttgaac tcatcatctt cctcaactgc ttcacggact acaaggacca 1500
ggggccccgc cgcgcagaga tccttgatga gatgcgagcg cacgtagaat cctggtggca 1560
ggaccaggtg cccagcctga gccttcagtt tcctgagcag aatgtgcctg aggctctgca 1620
gttccagctg gtgtccacag ccctgaagag ctggacggat gttagcctgc tgcctgcctt 1680
cgatgctgtg gggcagctca gttctggcac caaaccaaat ccccaggtct actcgaggct 1740
cctcaccagt ggctgccagg agggcgagca taaggcctgc ttcgcagagc tgcggaggaa 1800
cttcatgaac attcgccctg tcaagctgaa gaacctgatt ctgctggtga agcactggta 1860
ccgccaggtt gcggctcaga acaaaggaaa aggaccagcc cctgcctctc tgcccccagc 1920
ctatgccctg gagctcctca ccatctttgc ctgggagcag ggctgcaggc aggattgttt 1980
caacatggcc caaggcttcc ggacggtgct ggggctcgtg caacagcatc agcagctctg 2040
tgtctactgg acggtcaact atagcactga ggacccagcc atgagaatgc accttcttgg 2100

ccagcttcga aaacccagac ccctggtcct ggaccccgct gatcccacct ggaacgtggg 2160
ccacggtagc tgggagctgt tggcccagga agcagcagcg ctggggatgc aggcctgctt 2220
tctgagtaga gacgggacat ctgtgcagcc ctgggatgtg atgccagccc tcctttacca 2280
aaccccagct ggggaccttg acaagttcat cagtgaattt ctccagccca accgccagtt 2340
cctggcccag gtgaacaagg ccgttgatac catctgttca tttttgaagg aaaactgctt 2400
ccggaattct cccatcaaag tgatcaaggt ggtcaagggt ggctcttcag ccaaaggcac 2460
agctctgcga ggccgctcag atgccgacct cgtggtgttc ctcagctgct tcagccagtt 2520
cactgagcag ggcaacaagc gggccgagat catctccgag atccgagccc agctggaggc 2580
atgtcaacag gagcggcagt tcgaggtcaa gtttgaagtc tccaaatggg agaatccccg 2640
cgtgctgagc ttctcactga catcccagac gatgctggac cagagtgtgg actttgatgt 2700
gctgccagcc tttgacgccc taggccagct ggtctctggc tccaggccca gctctcaagt 2760
ctacgtcgac ctcatccaca gctacagcaa tgcgggcgag tactccacct gcttcacaga 2820
gctacaacgg gacttcatca tctctcgccc taccaagctg aagagcctga tccggctggt 2880
gaagcactgg taccagcagt gtaccaagat ctccaagggg agaggctccc taccccccaca 2940
gcacgggctg gaactcctga ctgtgtatgc ctgggagcag ggcgggaagg actcccagtt 3000
caacatggct gagggcttcc gcacggtcct ggagctggtc acccagtacc gccagctctg 3060
tatctactgg accatcaact acaacgccaa ggacaagact gttggagact tcctgaaaca 3120
gcagcttcag aagcccaggc ctatcatcct ggatccggct gacccgacag gcaacctggg 3180
ccacaatgcc cgctgggacc tgctggccaa ggaagctgca gcctgcacat ctgccctgtg 3240
ctgcatggga cggaatggca tccccatcca gccatggcca gtgaaggctg ctgtgtgaag 3300
ttgagaaaat cagcggtcct actggatgaa gagaagatgg acaccagccc tcagcatgag 3360
gaaattcagg gtcccctacc agatgagaga gattgtgtac atgtgtgtgt gagcacatgt 3420
gtgcatgtgt gtgcacacgt gtgcatgtgt gtgttttagt gaatctgctc tcccagctca 3480
cacactcccc tgcctcccat ggcttacaca ctaggatcca gactccatgg tttgacacca 3540
gcctgcgttt gcagcttctc tgtcacttcc atgactctat cctcatacca ccactgctgc 3600
ttcccaccca gctgagaatg ccccctcctc cctgactcct ctctgcccat gcaaattagc 3660
tcacatcttt cctcctgctg caatccatcc cttcctccca ttggcctctc cttgccaaat 3720
ctaaatactt tatataggga tggcagagag ttcccatctc atctgtcagc cacagtcatt 3780
tggtactggc tacctggagc cttatcttct gaagggtttt aaagaatggc caattagctg 3840
agaagaatta tctaatcaat tagtgatgtc tgccatggat gcagtagagg aaagtggtgg 3900
tacaagtgcc atgattgatt agcaatgtct gcactggata tggaaaaaag aaggtgcttg 3960

caggtttaca gtgtatatgt gggctattga agagccctct gagctcggtt gctagcagga 4020
gagcatgccc atattggctt actttgtctg ccacagacac agacagaggg agttgggaca 4080
tgcatgctat ggggaccctc ttgttggaca cctaattgga tgcctcttca tgagaggcct 4140
ccttttcttc accttttatg ctgcactcct cccctagttt acacatcttg atgctgtggc 4200
tcagtttgcc ttcctgaatt tttattgggt ccctgttttc tctcctaaca tgctgagatt 4260
ctgcatcccc acagcctaaa ctgagccagt ggccaaacaa ccgtgctcag cctgtttctc 4320
tctgccctct agagcaaggc ccaccaggtc catccaggag gctctcctga cctcaagtcc 4380
aacaacagtg tccacactag tcaaggttca gcccagaaaa cagaaagcac tctaggaatc 4440
ttaggcagaa agggatttta tctaaatcac tggaaaggct ggaggagcag aaggcagagg 4500
ccaccactgg actattggtt tcaatattag accactgtag ccgaatcaga ggccagagag 4560
cagccactgc tactgctaat gccaccacta cccctgccat cactgcccca catggacaaa 4620
actggagtcg agacctaggt tagattcctg caaccacaaa catccatcag ggatggccag 4680
ctgccagagc tgcgggaaga cggatcccac ctccctttct tagcagaatc taaattacag 4740
ccagacctct ggctgcagag gagtctgaga catgtatgat tgaatgggtg ccaagtgcca 4800
gggggcggag tccccagcag atgcatcctg gccatctgtt gcgtggatga gggagtgggt 4860
ctatctcaga ggaaggaaca ggaaacaaag aaaggaagcc actgaacatc ccttctctgc 4920
tccacaggag tgtcttagac agcctgactc tccacaaacc actgttaaaa cttacctgct 4980
aggaatgcta gattgaatgg gatgggaaga gccttccctc attattgtca ttcttggaga 5040
gaggtgagca accaagggaa gctcctctga ttcacctaga acctgttctc tgccgtcttt 5100
ggctcagcct acagagacta gagtaggtga agggacagag gacagggctt ctaatacctg 5160
tgccatattg acagcctcca tccctgtccc ccatcttggt gctgaaccaa cgctaagggc 5220
accttcttag actcacctca tcgatactgc ctggtaatcc aaagctagaa ctctcaggac 5280
cccaaactcc acctcttgga ttggccctgg ctgctgccac acacatatcc aagagctcag 5340
ggccagttct ggtgggcagc agagacctgc tctgccaagt tgtccagcag cagagtggcc 5400
ctggcctggg catcacaagc cagtgatgct cctgggaaga ccaggtggca ggtcgcagtt 5460
gggtaccttc cattcccacc acacagactc tgggcctccc cgcaaaatgg ctccagaatt 5520
agagtaatta tgagatggtg ggaaccagag caactcaggt gcatgataca aggagaggtt 5580
gtcatctggg tagggcagag aggagggctt gctcatctga acaggggtgt atttcattcc 5640
aggccctcag tctttggcaa tggccaccct ggtgttggca tattggcccc actgtaactt 5700
ttgggggctt cccggtctag ccacaccctc ggatggaaag acttgactgc ataaagatgt 5760

cagttctccc tgagttgatt gataggctta atggtcaccc taaaaacacc cacatatgct 5820

tttcgatgga accagataag ttgacgctaa agttcttatg gaaaaataca cacgcaatag 5880

ctaggaaaac acagggaaag aagagttctg agcagggcct agtcttagcc aatattaaaa 5940

catactatga agcctctgat acttaaacag catggcgctg gtacgtaaat agaccaatgc 6000

agttaggtgg ctctttccaa gactctgggg aaaaaagtag taaaaagcta aatgcaatca 6060

atcagcaatt gaaagctaag tgagagagcc agagggcctc cttggtggta aaagagggtt 6120

gcatttcttg cagccagaag gcagagaaag tgaagaccaa gtccagaact gaatcctaag 6180

aaatgcagga ctgcaaagaa attggtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtttaa 6240

tttttaaaaa gtttttattc ggaatccgcg 6270

<210> 16
<211> 1412
<212> DNA
<213> Mus musculus

<300>
<301> Coccia, E.M., et al.
<302> A full-length murine 2-5A synthetase cDNA transfected in NIH-3T3 cells
<303> Virology
<304> 179
<305> 1
<306> 228-233
<307> 1990
<308> NCBI/M33863
<309> 1993-06-11

<400> 16
ccaggctggg agacccagga agctccagac ttagcatgga gcacggactc aggagcatcc 60

cagcctggac gctggacaag ttcatagagg attacctcct tcccgacacc acctttggtg 120

ctgatgtcaa atcagccgtc aatgtcgtgt gtgatttcct gaaggagaga tgcttccaag 180

gtgctgccca cccagtgagg gtctccaagg tggtgaaggg tggctcctca ggcaaaggca 240

ccacactcaa gggcaggtca gacgctgacc tggtggtgtt ccttaacaat ctcaccagct 300

ttgaggatca gttaaaccga cggggagagt tcatcaagga aattaagaaa cagctgtacg 360

aggttcagca tgagagacgt tttagagtca agtttgaggt ccagagttca tggtggccca 420

acgcccggtc tctgagcttc aagctgagcg ccccccatct gcatcaggag gtggagtttg 480

atgtgctgcc agcctttgat gtcctgggtc atgttaatac ttccagcaag cctgatccca 540

gaatctatgc catcctcatc gaggaatgta cctccctggg gaaggatggc gagttctcta 600

cctgcttcac ggagctccag cggaacttcc tgaagcagcg cccaaccaag ctgaagagtc 660

tcatccgcct ggtcaagcac tggtaccaac tgtgtaagga gaagctgggg aagccattgc 720

ctccacagta cgccctagag ttgctcactg tctttgcctg ggaacaaggg aatggatgtt 780

atgagttcaa cacagcccag ggcttccgga ccgtcttgga actggtcatc aattatcagc 840

atcttcgaat ctactggaca aagtattatg actttcaaca ccaggaggtc tccaaatacc 900

tgcacagaca gctcagaaaa gccaggcctg tgatcctgga cccagctgac ccaacaggga 960

atgtggccgg tgggaaccca gagggctgga ggcggttggc tgaagaggct gatgtgtggc 1020

tatggtaccc atgttttatt aaaaaggatg gttcccgagt gagctcctgg gatgtgccga 1080

cggtggttcc tgtacctttt gagcaggtag aagagaactg gacatgtatc ctgctgtgag 1140

cacagcagca cctgcccagg agactgctgg tcaggggcat ttgctgctct gctgcaggcc 1200

catgacccag tgagggaggg ccccacctgg catcagactc cgtgcttctg atgcctgcca 1260

gccatgtttg actcctgtcc aatcacagcc agccttcctc aacagattca gaaggagagg 1320

aaagaacaca cgcttggtgt ccatctgtcc acctgttgga aggttctgtc tgacaaagtc 1380

tgatcaacaa taaaccacag caggtgccgt ca 1412

<210> 17
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense ODN (p40 subunit)

<400> 17
tttctgagat ccatcattga 20

<210> 18
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense ODN (p69 subunit)

<400> 18
tccccatttc ccattgc 17

<210> 19
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Control ODN sequence

<400> 19
gtctatgaat actttcctag 20

<210> 20

<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Control ODN sequence

<400> 20
cacctctatc tctctcg 17